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PROBLEMS OF DISEASES OF THE JOINTS*

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When one considers diseases of the joints, two pictures arise before one's eyes.

One is that of a boy aged about 20, immovable in bed because of severe pain in his swollen joints, high temperature and palpitation of the heart. After some days or several weeks, the pain and swelling of the joints will disappear perfectly and he will recover, but a mitral incompetence remains and he will be a lifelong invalid. Several years later, a second or third attack of rheumatic fever appears, and perhaps at last a recurrent endocarditis brings on death with the symptoms of a septic disease.

The second picture is that of a woman in an almshouse. For many years, she has suffered from pain in the fingers, wrists, knees and other joints. Most of the joints become more and more stiff and manifest deformities of all kinds. The whole day long she sits in an armchair or lies in bed. She is unable to move her deformed hands, to take a spoon or glass to her mouth, to bring a handkerchief to her nose or to brush a fly away from her face. She is completely dependent on the good will of her nurse; she is forgotten by her family and friends. Month after month passes in the same solitude, though she is contented as long as her pains do not grow excessive. It is interesting to study the mental attitude in these chronic diseases.

One might think that both these forms were different stages of the same disease, the acute and the chronic form, but an exact study, especially of the pathologic anatomic lesions, shows that these diseases of the joints are of a different character.

SYMPTOMS AND SIGNS

The student of pathologic changes of diseases of the joints will do best when he follows the steps of the great masters, and this is necessary for all those who are not pathfinders themselves. In the early dawn of exact pathologic study, Virchow and Volkmann taught

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students to distinguish those diseases of the joints which are of inflammatory character and those in which only the degenerative processes in the joints prevail.

The classic signs of inflammation are found in the acute diseases of the joints. The old Roman, Celsus, characterized inflammation as a syndrome consisting of the following symptoms: rubor, calor, tumor, dolor and functio læsa. The inflamed joint is hot and red from the increased flow of blood; it is swollen through exudation into the cavity of the joint and its surrounding tissues; the joint fluid is rich in protein and leukocytes, and the joint is extremely painful and therefore resists attempts on the patient's part to move the affected limb.

In the degenerative form, only slight pain is present; there is no exudation but, instead, a slow degeneration of the cartilage takes place, accompanied by a proliferative outgrowth of osseous tissue.

In accepting this distinction between inflammatory and degenerative proliferative processes, it must be realized that only the acute forms of inflammation present a purely exudative character and that in nearly all cases of inflammation some signs of regeneration are present.

Following the destruction of the tissue in a severe inflammatory process of the joint, repair takes place through the proliferation of connective tissue cells. This process often results in a thickening and retraction of the capsule and in hypertrophy of the synovial membrane. Ofter ankylosis or adhesions take place between contiguous surfaces, and in some instances large masses of new bone are produced.

In the acute arthritis of rheumatic fever, the signs of inflammation are especially pronounced in the synovial membrane. In healthy joints, the latter is a smooth, undifferentiated layer, poor in cells and in blood vessels, which never come to the surface, but which are rich in nerve fibers. In cases of acute rheumatic arthritis, redness and edema of the synovial membrane extending into the periarticular connective tissue are demonstrable at biopsy. The synovitis is characterized particularly by a perivascular and perineural infiltration of leukocytes and mononuclear cells. Homer Swift believes that the intensive articular pain in rheumatic fever is due to the pressure of the inflammatory exudate on the nerves. The cartilages are completely free from blood vessels and nerve fibers, and therefore cannot play an active part in the inflammation or the pain.

Roentgen-ray studies have shown that in all cases of inflammatory disease of the joints the bony framework of the epiphysis is sooner or later greatly diminished and the epiphyseal ends of the bones suggest a cavity. This atrophy of the epiphyseal regions is characteristic of all acute and subacute diseases of the joints and affords a characteristic distinction between the inflammatory and the productive hypertro-

phic processes. The origin of this atrophy is not yet perfectly understood. It may be that it is due mainly to the inactivity of the painstricken joint. Indeed, the maintenance of the normal shape of the cartilages and other joint structures is dependent on activity and pressure. As soon as a joint becomes immobile, some degeneration of the cartilages takes place and the osseous matter of the bones decreases. However, in some cases that I have seen a protracted immobilization of a normal joint resulted in only slight atrophy of the bones but produced a severe retraction and fixation of the capsule. In the atrophy caused by inactivity, the spaces of the bone marrow between the atrophic trabeculae are filled with fat as in normal bones. But in the cases of acute inflammations of the joints I have found great infiltrations of lymphocytes and polymorphonuclear leukocytes filling the bone marrow spaces adjoining the affected joint. This cellular proliferation produces a dense border surrounding the bony trabeculae. Often a zone of osteoid tissue is found at the periphery of this inflammatory process. It may be assumed that the rarefied areas seen in roentgenograms of the epiphyseal ends of the bone are the end-results of the thinning and resorption of the trabeculae incident to the inflammatory reaction. I have reason to believe that the infectious process does not begin in the synovial membrane but in the bone marrow of the epiphysis, which is recognized as rich in blood vessels. The infection may have its origin in the bone marrow of the epiphysis and may extend from that point to the periosteum and synovial membrane, as is the rule in osteomyelitis and tuberculosis of the joints.

Dr. Kyes of Chicago agrees with this point of view and from his experiments with animals concludes that in general bacteremias the micro-organisms localize especially in the spleen and in the epiphyses of the bones, because in those regions the rate of the blood stream is slow. A. Fraenkel and his co-workers in Hamburg have often isolated micro-organisms from the bone marrow of the vertebrae and the bone marrow of other regions in a large proportion of the cases of sepsis that they studied. Furthermore, Wassermann believed that the production of antibodies takes place especially in the epiphyseal osseous spaces.

The inflammatory process which has progressed from the epiphysis to the periosteum now extends to the synovial membrane, and the exudation into the cavity of the joint takes place in most cases. This exudate is in most cases free from bacteria, but despite this fact, one should not conclude that the inflammation is not of an infectious character. In erysipelas and in many cases of pleurisy, the exudate is also free from bacteria, but it is well established that the bacteria lie in the inflamed membranes. However, in gonococcal arthritis, the joint exudate is often rich in gonococci.

ARTHRITIS

The word rheumatism is derived from the Greek word rheo, to flow, or rheuma, flowing. This expression implies that a noxious substance flows through the body and acts in many distant regions. This flowing can take place only through the blood or lymph channels.

The term rheumatism has been applied to a great many pathologic conditions, myalgias, neuralgias and arthralgias. I propose here to limit my discussion to a general pathologic anatomic study of the true inflammatory and degenerative-proliferative diseases of the joints.

Secondary arthritis is frequently seen in the course of many infectious diseases such as scarlet fever, dysentery, gonorrhea, cerebrospinal menignitis, puerperal fever and Malta fever. Gerhardt grouped those arthritides of obvious metastatic character under the term, "Rheumatoide Erkrankungen," and he emphasized particularly the tendency toward secondary arthritis in cases of putrid bronchiectasis.

An obstinate type of polyarthritis is sometimes associated with bacillary dysentery. It is still a debatable question as to whether the inflammation of the joints found in this disease is caused by the toxin of the bacillus of Shiga-Kruse or by a secondary infection with streptococci which have been found in the affected joints.

A painful arthritis is frequently seen in the course of scarlet fever. Since the swelling and tenderness in the joints first manifest themselves on about the tenth day of the disease and begin to decrease in intensity in a few days, the picture is not unlike that found in the serum sickness occurring in the first week following the intravenous administration of large quantities of foreign serum in the treatment of diphtheria, tetanus, or pneumonia type I. True serum sickness is characterized by the presence of fever, urticaria, transient leukopenia followed by leukocytosis, eosinophilia, and by an arthritis of varying intensity. It is tempting to conjecture that the arthritis seen in rheumatic fever is an allergic phenomenon paralleling that seen in serum sickness. In this connection, it is of interest that Boots and Swift have demonstrated that the joint exudate in rheumatic fever is microscopically indistinguishable from that occurring in serum sickness.

Besides the mild arthritis of scarlet fever already described, there is a severe type which comes on in the later stages of the disease. This has been shown to be due to streptococcus metastases.

One of the clearest examples of inflammation of the joints is seen in gonorrheal arthritis. It occurs nearly always in conjunction with an acute gonorrheal infection. Relapses of the arthritis are rare, but they sometimes occur as the sequelae of a new infection with the gonococcus or an acute resumption of activity on the part of a previously quiescent gonococcal focus. There is abundant evidence that the latter process

takes place frequently. It is particularly apt to occur during the puerperium or following excesses in Baccho et Venere, without reinfection, however. I am acquainted with a physician who suffered three severe relapses of gonorrheal rheumatism lasting many weeks. During the arthritis, a tenosinovitis of both hands ensued, which resulted in a bilateral shortening of the fascia palmaris. When the inflammation had ceased, both hands presented the picture of severe Dupuytren's contracture, which greatly hindered the physician in his practice. Painful gonococcal inflammation also occurs in the achilles tendon, and for the most part achillodynia is due to gout or to the gonococcus.

Typical gonorrheal arthritis begins with a high temperature and with inflammation in many joints, but in a short time the majority of the inflamed joints appear to have undergone healing. The process then becomes localized in the knee joint, the ankle joint, or in many of the small joints of the hands, producing almost unbearable pain and disability over a long period of weeks. Roentgenograms of the involved joints begin to show significant foci of rarefaction in the epiphyseal portion of the bones at the end of the second week of the disease. One cannot differentiate these roentgenograms from those seen in acute rheumatic polyarthritis.

In some cases of gonorrheal arthritis, an endocarditis and sometimes a myocarditis occurs. There can be no doubt that this is a symptom of a definite bacterial metastasis, since Leyden and others have isolated the gonococcus in pure culture from the valvular vegetations in endocarditis and from purulent exudate in the joints.

A distressing ankylosis often remains as a sequel to the gonococcal inflammation of the joints. This must be combated early in the disease by a regimen of persistent active and passive motion. Although the impairment of motion of the joints may be due to a simple thickening of the capsule, the usual picture in the late stages of gonorrheal arthritis is that of fibrous adhesions and osseous ankylosis between the adjoining joint structures. A destruction of the cartilages goes hand in hand with this process.

Degeneration and destruction of cartilages of the joints are sometimes seen in other types of severe infectious arthritis. I remember particularly one case of multiple involvement of the joints of the upper and lower extremities which was due to *B. coli* acting its frequent rôle of a secondary invader to a gonococcal infection of the prostate and seminal vesicles. Following the ascending infection of the urinary tract, the subacute polyarthritis ensued and thereafter the patient died.

In Malta fever, polyarthritis is a frequent and characteristic symptom. In this disease the involvement of the joints runs a benign,

though chronic, course. The pneumococcus, meningococcus and staphylococcus are occasionally the causes of severe inflammation of the joints, but the commonest organism that is found in the acute polyarthritis is the streptococcus.

Singer of Vienna believes that the streptococci form one homogenous group, but, through the work of Schottmüller of Hamburg and Brown, working at the Rockefeller Institute in New York, it has been proved that there are varieties of the micro-organism. Schottmüller has described four types of streptococci:

1. *Streptococcus non-hemolyticus,* which grows in short chains, does not hemolyze blood on the blood agar plate and appears frequently as a saprophyte in the nasopharynx. According to some investigators, it is often found in the joint exudate, blood and pericardial exudate of patients with rheumatic fever.

2. *Streptococcus hemolyticus,* which grows in long chains, hemolyzes the blood on blood agar plates and is usually of a higher virulence than 1.

3. *Streptococcus viridans,* which grows in short chains. On blood agar plates, instead of producing hemolysis, it becomes surrounded by a greenish zone of variable diameter.

4. *Streptococcus putrificans,* which is a small coccus that is difficult to cultivate under ordinary laboratory conditions. With appropriate anaerobic environment, growth takes place with the production of an ill-smelling odor.

The latter organism is sometimes found in the sepsis following abortion, and Rosenow believes that it is this coccus, so often found in the apexes of infected teeth, which is the etiologic factor in some of the arthritides. In Rosenow's laboratory, I have seen metastatic arthritides follow the intravenous injection of such anaerobic streptococci into dogs.

Streptococcus viridans is found frequently in the crypts of inflamed tonsils and also at the apexes of infected teeth. In comparison with the hemolyticus, it possesses a slight degree of virulence. It is astonishing, however, that the supposedly mildly virulent viridans, which is apparently a frequent inhabitant of the human pharynx, can so easily pass into the blood stream and produce an endocarditis and sepsis which heal only rarely. This clinical picture, the endocarditis lenta of the European school and the subacute bacterial endocarditis of the American students of the disease, is characterized by chronic mild fever, progressive anemia accompanied by a leukocytosis and occasional showers of large mononuclear cells, sometimes an enlarged spleen, changing cardiac murmurs, cachexia, positive blood culture and many embolic phenomena. Particularly frequent is the embolic glomerulonephritis which occurs late in the course of the disease. The body seems unable to offer any significant resistance in the form of immune bodies against this comparatively mildly virulent coccus, which hardly ever produces a purulent process.

An important but unsettled problem is the question as to whether a mutability of these strains of Schottmüller takes place. Will *Streptococcus hemolyticus,* which has maintained is identity during cultivation under one set of conditions, change its characteristics to resemble those of a viridans, if the culture medium is changed, if the carbon dioxide tension is increased, or if the immune substances in the body in which it is growing are modified? More facts on this subject will have great significance for the pathologic study of the future.

Streptococcic infections often are the inciters of transient or persistent types of arthritis which closely resemble those seen in rheumatic fever. Inflammatory disturbances due to the streptococcus, with or without an accompanying arthritis or endocarditis, may originate in many parts of the body. Paessler of Dresden pointed out that the majority of those foci of infection are found in hollow spaces such as the gallbladder, urinary tract or thrombosed veins, or in semiquiescent abscesses which are not in direct relation with the living cells or with the circulating fluids of the body. It is not an uncommon experience to observe cases in which streptococci, having remained latent in such foci over a long period of years, suddenly invade the blood stream, producing a chill or fever. This picture is frequently seen in cases of septic thrombi, which often follow slight wounds, operation or unsterile intramuscular injections. But unquestionably the most important foci for the dissemination of the cocci are the tonsils and the infected apexes of carious and devitalized teeth.

RHEUMATIC FEVER

When one leaves the field of the known etiologic factors in acute disease of the joints, one is struck by the fact that it is difficult to establish at the bedside a sharp line of classification that will differentiate between an arthritis due to a proved streptococcic infection and that caused by rheumatic fever. In fact, there are so many apparently transitional cases between true rheumatic fever and the mild cases of streptococcic sepsis that one must consider the possibility that rheumatic polyarthritis is only an attenuated form of strepococcic sepsis.

Jürgensen of Tübingen was the first to recognize rheumatic fever as a true infectious disease. In his further studies he found that a great many cases that he had previously believed to be rheumatic fever were in reality cases of sepsis caused by a cryptogenetic array of microorganisms. A group remained in which no causative agent for the polyarthritis could be found.

Undoubtedly, rheumatic fever is an infectious disease. Fiedler was one of the first observers to call attention to tonsillitis as a primary focus of infection for polyarthritis. This observation of Fiedler and of many

others has led to the approved procedure of extirpation of inflamed tonsils in patients with rheumatic fever as a prophylactic measure against subsequent lapses. Like many other observers, I have seen rapid recovery and cessation of relapses following the removal of infected tonsils in cases of rheumatic fever. But in many other such cases, tonsillectomy failed to modify the normal course of the disease. However, it is incorrect to assume that the tonsils are the only source of the infecting agent. Schottmüller has clearly demonstrated that in many such cases there may be secondary foci of infection in the veins or in the valves of the heart as well as in the tonsils. It follows naturally, therefore, that as little therapeutic result can be hoped for by the removal of only the infected tonsils in rheumatic fever when there are other active areas as might be expected from the extirpation of a chancre after the generalized distribution of the spirochete has taken place.

Experience has taught me to advise against tonsillectomy until all signs of tonsillitis have disappeared. When this warning has not been heeded, I have often seen an aggravation of the patient's illness, high temperature, and a pronounced albuminuria and hematuria. It would seem as if through the operative procedure bacteria were discharged into the circulation and thence to the kidneys. The same warning holds true for operations on the acutely inflamed nasal accessory sinuses, for I have seen cases in which sepsis and death followed too great an enthusiasm for early surgical intervention.

Typical rheumatic fever has been classified as a separate disease entity by McCallum, Hegeler and many others. Homer Swift has defined rheumatic fever "as a disease of undetermined etiology, characterized by the febrile state, by migratory inflammations of structure covered by serous membranes, by a peculiar inflammation of the myocardium and finally by the tendency for the febrile symptoms and arthritis to disappear following adequate doses of salicylates."

The disease begins most frequently between the tenth and the thirtieth year, and the great majority of patients have their first attack in the second decade. It is noteworthy that the younger the patient the greater the liability to endocarditis and chorea. This greater susceptibility to complications in the earlier years of life would seem to rest on the possibility that tissue reactions are a function of their age. Perhaps the richness in cells of the rapidly growing epiphysis increases the tendency of youth toward the infectious diseases of the bones and joints, such as tuberculosis and osteomyelitis.

Payr has argued that constitutional factors are important in the occurrence of rheumatic fever since there are so many instances of more than one case in the same family, as has been shown by Pribram,

St. Lawrence, Faullener and White, Draper and others. However, St. Lawrence has shown from a parallel study of cases of rheumatic fever and tuberculosis that the conditions are of about the same magnitude in families. Since it is known that tuberculosis is an infectious disease, it might be argued that rheumatic fever is of the same nature, and it would be necessary to involve such factors as constitution to explain the high incidence of the disease in families.

However, many patients who have gout give a history of pains in the joints in their youth. I was previously of the opinion that this youthful condition was an early polyarticular form of gout. Experience taught me, however, that my previous belief was incorrect, for most of the patients who had gout, and who had suffered polyarthritis in their youth, had in reality passed through a siege of real rheumatic fever at that time. Perhaps one might predicate that in such cases there is a constitutional weakness of the joint structures, which in youth predisposes the patient to rheumatic fever and in later life to gout.

Furthermore, the occurrence of chorea with rheumatic fever is characteristic. It is noteworthy that this nervous complication practically always takes place in the second decade of life or in the first pregnancy of young women. It is then nearly always combined with a benign endocarditis.

Characteristically, the inflammatory process in the joints takes the form of a rapidly migrating polyarthritis. When one joint has apparently recovered, the infection may suddenly appear in another one After a variable period of time, the swollen, painful joint will again approach its normal condition, and the complete restoration of function proves that the cartilages have remained intact. The inflammatory process is not limited to the synovial membrane, for the pronounced exudative and proliferative process also involves the periarticular connective tissues.

Unlike many other infectious diseases, rheumatic fever is characterized by a marked tendency toward relapses, which may possibly be due to the persistence of a latent focus of infection. It may take many years for a relapse to occur, for Mackie, at the Presbyterian Hospital in New York, has recently shown that "only 57 per cent of the first recurrences were found to develop within a period of four years following the first attack of rheumatic fever." Fiedler, Paessler and others have reported that in from 20 to 80 per cent of first attacks or relapses of rheumatic fever there was an associated tonsillitis. In my studies, this figure is about 50 per cent.

One of the frequent accompaniments of rheumatic fever is valvular endocarditis. Mackie found that there was a serious cardiac involve-

ment in 68 per cent of his 393 cases of rheumatic fever, irrespective of age. Other reports have placed the incidence nearer 35 per cent. However, Cohn and Swift have concluded from careful electrocardiographic studies that in thirty-five out of thirty-seven cases of rheumatic fever which they followed, the heart was involved in one way or another.

The pathologic condition in rheumatic endocarditis has been clearly described by McCallum, Libman and others. The mitral valve is most frequently involved; and the aortic is next in order. It is not an infrequent occurrence to find tricuspid or pulmonary rheumatic valvulitis. The pathologic picture of rheumatic endocarditis stands in marked contrast to *Streptococcus viridans* endocarditis. In the latter, large, soft, bead-like excrescences are found on the valves, the chordae tendineae and sometimes on the contiguous mural endocardium. The process in streptococcic infection is essentially of an ulcerative, destructive character. Minute aneurysms of the valves, leading to perforations, and friable, divided chordae tendineae are not uncommon in streptococcic endocarditis. In contrast to this picture, rheumatic endocarditis is characterized by a thickening due to cellular infiltration of the valves, sometimes followed by a proliferation of fine, wart-like verrucosities along the lines of closure of the valves. This process may proceed to a thickening and union of the valve cusps, and finally the valve orifice may so contract through the proliferative reaction as to produce a serious impediment to the natural course of the blood stream. McCallum has recently shown that the rheumatic inflammation frequently involves the mural endocardium of the left auricle. A curious whitish thickening extends from the posterior leaflet of the mitral valve along the auricular endocardium.

The myocarditis of rheumatic fever, however, is characterized by the presence of many noduli, which were first described by Aschoff. These cellular foci are found most frequently in the myocardium of the left ventricle in the vicinity of small arterioles. In the center of the nodule is a tiny area that takes stains poorly. It probably contains necrotic tissue and bits of fibrin. Clustered around this point in a thin meshwork of young fibroblasts, often in spindle-shaped form, is a collection of large cells containing one or many pale vesicular nuclei. Surrounding these so-called epithelioid cells is a zone containing lymphocytes, plasma cells, and occasional polymorphonuclear cells. Swift has described lesions of a similar nature in the synovial membrane of inflamed joints in rheumatic fever. He also believes that accumulations of cells reminiscent of Aschoff bodies may be made out in the subcutaneous nodules often seen in this disease.

Swift and other observers have felt that the response to the administration of salicylates is a characteristic feature of rheumatic fever. Many physicians will not agree with this point, for in many cases of severe rheumatic fever the salicylates have slight, if any, effect on the relief of pain and on inflammation of the joints. In such cases, I have sometimes observed that better results follow the administration of such pyrazalon derivatives as melubrin. A second proof that the salicylates do not act specifically against the cause of rheumatic fever is that they have no influence on the development of carditis, the liability of relapse, and the duration of the disease. Finally, the salicylates sometimes produce splendid therapeutic effects in gout, which obviously is unrelated to rheumatic fever.

Careful bacteriologic investigations have failed to disclose a specific etiologic agent for rheumatic fever. Schottmüller, Hegeler, McCallum and others maintain that the cause of the disease is unknown and that when a micro-organism is found in the blood stream or exudates of a patient with rheumatic fever it must be considered a secondary infection. It is true that when a bacterium, usually a streptococcus, is found in the blood of a patient who has previously had rheumatic fever, the clinical picture is usually that of endocarditis lenta. McCallum holds that the valves which have been thickened and shortened by the rheumatic fever process offer a point of vantage for further infection by secondary invading micro-organisms.

Although the pathologic picture of the endocarditis in rheumatic fever is so unlike that found in sepsis, in which a definite micro-organism is undoubtedly the causative factor, the clinical picture and course of rheumatic fever, with its early tonsillitis followed by inflammatory manifestations in such distant foci as the joints, the three layers of the heart, the pleura, etc., strongly suggest that an infectious agent has invaded the blood stream. In this regard, Clawson of Minneapolis and Small of Philadelphia have recently presented evidence that adds further plausibility to the fact that an anhemolytic streptococcus may be frequently present in the blood stream of patients with simple rheumatic fever.

Schottmüller has recently shown that the bacteria in the blood stream in cases of sepsis either are rapidly destroyed through the bactericidal properties of the blood serum, or are quickly taken up by the tissues of the body. A persistent bacteremia occurs only when the defensive mechanisms of the body have been reduced to a minimum. Such a condition does not, however, signify that the bacteria are growing in the blood, for their multiplication may be due to the fact that they are rapidly swept from the endocardial vegetations in which they are presumably growing luxuriously.

BACTERIOLOGIC INVESTIGATIONS

In recent years, Oeller, and Dietrich and Sigmund, his pupil, have studied the fate of bacteria which have reached the blood stream in disease processes and through experimental means. These workers found that when virulent micro-organisms were injected intravenously into animals, they were not demonstrable in the circulating blood after a relatively short period of time. Their rapid disappearance from the blood stream was due in part to the destructive action of the bactericidal substances of the blood, which were both specific and unspecific; in part also to the phagocytic action of the endothelial cells of the capillaries and venules. Shortly after the injection, the micro-organisms were found to have been carried into the adventitial spaces of the vascular channels. In the later stages of such cases, Dietrich and Sigmund showed the development of peculiar perivascular nodules which were made up in part of cells rich in protoplasm. These cells are the histiocytes, or the large macrophages of Metchnikoff, which are derived from the reticulo-endothelial system. Interspersed among the large wandering cells are polymorphonuclear leukocytes and lymphocytes. In the nodule, which bears a resemblance to a tubercle, the micro-organisms may undergo destruction through the agency of the cellular phagocytic elements. Roessle conceives of the inflammatory reaction as mainly a digestive process of the invading foreign bodies. Many years ago, I pointed out the digestive properties of the leukocytes and of the exudate in pneumonia.

It can be concluded that the absence of bacteria in the blood is therefore not a reliable sign that an invasion of the bacteria has not taken place at a previous time.

The little perivascular nodules described by Dietrich and Sigmund in such organs as the liver, spleen and lung manifest a striking histologic resemblance to the Aschoff bodies seen in rheumatic fever. If, furthermore, such "rheumatic nodules" can be demonstrated in severe forms of scarlet fever, those nodules cannot be considered as a pathognomonic sign of the specific nature of rheumatic fever.

In regard to the defensive mechanisms of the body against disease, the work of Theobald Smith on natural immunity is of great importance. He has shown that if new-born calves are deprived of the mother's first milk and therefore the colostrum, they succumb in a few days to a generalized *B. coli* septicemia. If, however, the calves are allowed to drink the colostrum immediately after birth, the *B. coli* sepsis does not take place, because the acquired immune bodies prevent the passage of the bacteria through the intestinal wall. If, on the other hand, the calves are not allowed to drink the colostrum

until a few days after birth, the delayed administration of protective substances gives them only an incomplete immunity and the animals develop a purulent polyarthritis. This is another example of the predilection that bacterial metastases have for the tissues of the joints.

The unspecific immunity with which the individual is endowed at birth, as well as the acquired immunity of the organism, may be so great that the invading bacteria are overcome at their entrance, and then the bacterium is avirulent for the body. In more serious forms of the disease, the invading power of the micro-organism is such that it passes into the blood stream and other tissues. The resisting forces of the individual may soon succeed in localizing the process, overcoming the invaders and causing a healing process. In the severest forms of infection, the immune agents of the body are unequal to the task of conquering the foe. The micro-organisms multiply in all the tissues, and the condition is described as sepsis.

This failure of the body to cope with the invading virus can be explained in two ways. It may be first argued that the micro-organism is possessed of particularly malignant toxic qualities. On the other hand, the results of animal experimentation and clinical observations have shown that the powers of immunity of a person may be seriously hampered or changed by certain poisons, roentgen-ray injuries, pregnancy and the effect of accompanying diseases, such as diabetes. One should therefore conceive of the virulence of a virus as dependent not only on the specific nature of the micro-organism but also on the powers of resistance of the body. It is to be emphasized that the term "virulence" is a relative matter, for a person who has long overcome a specific infection may finally succumb to it, since the effects of exhaustion or other diseases have produced a breakdown of resistance.

Complete healing takes place in many cases of rheumatic fever without any changes in either the joints or the valves of the heart. In other cases, the valves may become shortened and thickened and may remain in this fashion throughout the patient's life if there are no relapses. In some cases, however, the inflammatory process in the involved joint does not heal completely but persists as a mild, subacute or chronic polyarthritis. In the further development of such cases, periods of variable duration, characterized by tonsillitis, pain in the joints and fever, may occur. There is the possibility that each of these attacks records the entrance of a new crop of bacteria into the blood stream. In the later stages of this relapsing type of rheumatic fever, the symptoms in the joints fall into the background, and the clinical picture slowly begins to simulate that of a mild sepsis. Soon the temperature remains persistently high, the patient develops the profound pallor so aptly described as "café au lait," and the changing heart

murmurs suggest that an active inflammatory process is taking place in the valves of the heart. The splenic tumor may sometimes be felt, and if blood cultures are carefully taken in the periods of highest fever a micro-organism, usually a streptococcus, may be recovered. Characteristic showers of emboli are thrown off from the ulcerated, infected valves of the heart. This leads to the production of multiple infarcts, especially in the spleen, kidneys and brain.

Is it really necessary for one to assume that in such cases a secondary invader of a particularly malignant character has engrafted a new infection on the valves already damaged in the inflammatory process of rheumatic fever? I believe that the slowly progressive history of such patients offers no basis for the opinion that the septic process is a secondary infection. I am of the opinion that the terminal process merely represents the results of the collapse of the resistive power of the individual against the same micro-organism which has been in his body for a long series of years. One must, therefore, consider the possibility that the infective agent, usually a streptococcus, which produces the terminal endocarditis, is the same as that which caused the repeated attacks of rheumatic fever in the patient's earlier life.

However, the data at hand are too meager to cause a denial of the possibility that a secondary infection with the streptococcus may not implant itself on the ground prepared for it by the unknown cause of the rheumatic fever in much the same fashion in which secondary *B. coli* infections follow the inflammatory tract paved by the gonococcus.

ACUTE ARTHRITIS

Another problem in general pathology of the joints is that of acute arthritis produced by toxic agents. It will be seen that the same type of destruction and ankylosis is present in the severe arthritides of gouty origin as in the pronounced cases of infectious arthritis.

In gout also, the attacks in the joints are of a true inflammatory nature. Exudation into the cavity of the joints is not uncommon, and in some cases this exudate was found rich in sodium urate, so rich in fact, that the supersaturated fluid withdrawn by puncture forms an abundant precipitate of fine crystals in needle-like form in the watch glass. There can be no doubt that the presence of the sodium urate solution is the source of the inflammatory reaction. I do not know how this accumulation of sodium urate in the joints takes place. Perhaps its source is the synovial membrane or the blood vessels of the subchondral epiphysis. The deposit of urate crystals is found on the surface of the cartilage covered by a thin layer of epichondrium.

Brogsitter, of this clinic, has shown that the sodium urate crystals are especially localized in little cup-like, punched out cavities of the

cartilage. The urate deposits produce a local necrosis and destruction of the cartilage, which undergoes a remarkable type of disintegration and change in character. In the lower layers of the affected tissue a zone of calcareous deposit becomes visible, and young blood vessels and bone marrow cells grow into this primary calcification to produce a wart-like, osseous outgrowth. This new formation of bone is seen whenever destruction of cartilage occurs.

The diagnosis of a gouty condition of the joints often causes difficulty, because sometimes, especially in younger patients, the disease picture is primarily that of polyarthritis accompanied by pyrexia. In these cases of polyarticular gout, the diagnosis remains in doubt until

Appearance of hands in a case of gonorrheal arthritis, showing the characteristic hollow spaces of the epiphyses.

the characteristic inflammation of the big toe appears or the blood uric acid value is abnormally high. In elderly patients, the clinical picture of gout may closely resemble that of arthritis deformans, but in most of these questionable cases the chemical examination of the blood serum determines the diagnosis.

It has been shown that an acute inflammation of the joints can occur in toxic as well as in infectious diseases. I shall now describe the chronic inflammatory process, but before entering into this discussion it will be necessary to define the expression "chronic inflammation."

"Chronic inflammation" describes an inflammatory process of long duration and not the permanent, inactive residue of an acute process.

Scar formation between two bones after an acute gonorrheal arthritis is not a chronic arthritis in the same sense that a mitral stenosis is not a chronic endocarditis. A chronic inflammation is therefore a processus and not a status. It is always the product of a long standing irritative agent. How can one explain the long duration of inflammatory processes of infectious or toxic origin?

I think it can be assumed that an acute inflammation is the product of a strong and vivid reaction of tissues against a noxious agent. This violent, acute reaction may result in complete recovery, but in some cases healing takes place with a permanent alteration in structure.

A chronic inflammation, however, is in most cases a mild reaction of long duration, which may be explained in two ways. The long drawn out struggle between the host and the invader is due to the fact either that the host is unable to produce a rapid reply to the invader or that the latter is so weak that it damages the host only to a slight extent. I believe that a combination of both these explanations should come into consideration.

The chronic infectious inflammations of the joints are nearly always characterized by slight elevations of temperature when a fresh involvement appears in a new joint. Relapses are frequent, but the attacks are rarely of a severe character. Sometimes the etiologic focus of the infection can be located in the tonsils, teeth, accessory nasal sinuses, appendix, or the genital tract. Elimination of the source of such foci of infection is not always possible, and even when the primary inflammatory area is disposed of, the progress of the arthritis is not necessarily arrested.

The chronic diseases of the joints of inflammatory nature are always characterized by a distinct atrophy of the epiphyseal osseous framework, and in some cases the diaphysis is involved in the same process. My own microscopic studies have not yet answered the question as to whether this atrophy is due only to inactivity or to chronic inflammatory processes in the bone marrow. In some of these cases, it has been possible to make out tiny foci of lymphocytic infiltration in the bone marrow. However, there is the possibility that the immobility of the limb which has caused the atrophy of the muscles may have caused the same changes in the bones discussed above.

It is interesting to observe that in true chronic infectious arthritis new osseous formations are seldom seen. Goldthwait is correct in his differentiation between the atrophic and hypertrophic forms of diseases of the joints. The chronic diseases of the joints of infectious origin belong, nearly always, to the atrophic type, whereas the chronic forms of gout are mostly of a hypertrophic character. Frequently, the cases of chronic infectious arthritis progress to a state of deformity and fixation of the joint.

The chronic infectious type of polyarthritis is frequently accompanied by some pathologic change of the heart or kidneys and is a serious condition. Continuous pain and progressive stiffness are present, and in later years anemia and cachexia may be prominent features. Among the chronic progressive inflammatory diseases of the joints is a type that begins acutely or subacutely, often runs a course characterized by relapses, and then shows a transition to a subacute polyarthritic form.

Another type begins as a chronic disease with mild swellings and pains of the finger joints, "doigts en radis," without a rise in temperature, and then slowly extends over more and more joints, deforming and stiffening them. I am not at all certain as to whether this second form is also of infectious character, and in most of these cases the origin of the disease remains completely in the dark. I must confess that this frequent form of the disease is the darkest part of the whole question.

I have seen cases in which the disease has affected nearly all the joints during the course of many years, but no signs of a severe alteration of the cartilages were demonstrable by the roentgen ray or autopsy. The mobility of the joints was slightly impaired, but the bones of the hands, feet, arms and legs were extraordinarily atrophic and showed only faint lines in the roentgenograms. The atrophy extended to the muscles, and the hands became transformed into useless, soft appendages. There were no signs of an inflammatory character or of osteomalacia. An anatomic preparation of the bones of such a hand was as light as a feather. I was at a loss to account for the etiology of this peculiar disease. I have seen two such cases in one family, so that perhaps constitutional factors are of importance in determining the susceptibility to the disease.

Among the chronic progressive diseases of the joints, one has also to consider those which are of toxic origin. The best known are ochronosis and gout. In ochronosis, the work of Virchow, Sir William Osler and other authors has shown that an autointoxication with dioxyphenylacetic acid leads to a brown discoloration and slow degeneration of all the cartilages of the body, including those of the ears. In Virchow's original case, the ligaments and fibrous tissues were of a brownish black. In connection with the degeneration of the cartilages, the epiphyseal portion of the bones may hypertrophy as in arthropathia deformans.

Among the metabolic disorders, chronic uratic gout also can produce destruction and hyperplastic processes of the joints. It is sometimes difficult to find the characteristic accumulations of sodium urate. Munk believes that the hollow spaces shown by the roentgen ray in the epiphyses are characteristic of chronic gout, and our own anatomic

preparations have shown that these hollow areas in the epiphyses are filled with sodium urate and are surrounded by a mass of inflammatory cells. However, with Krebs, I have found similar hollow spaces in the epiphyses of chronic infectious arthritis in patients who have never had gout, and therefore these roentgen-ray examinations cannot be considered a reliable diagnostic feature of gouty accumulations.

It is astonishing to observe that there is less pain and inflammation in the chronic than in the acute attacks of gout. In chronic gout, huge concretions of urates may be present without producing either pain or inflammation. At one time it was thought that in chronic gout the tissues had become so accustomed and insensible to uric acid that sodium urate was no longer able to produce an inflammatory reaction. I tried to confirm this hypothesis by observing the results of subcutaneous injections of sodium urate. It was found that these injections produced the same degree of inflammation and pain in acute and chronic cases of gout, and more or less degree of inflammation in healthy, nongouty persons.

I was unable to detect a diminished reactibility or hypersensibility of the gouty patients to these injections of sodium urate.

Chronic cases of gout sometimes produce hyperplastic processes of the bone near the cartilages. These excrescenses on the fingers are the well known nodes of Heberden. This new formation of osseous tissue proximal to the injured cartilage is of great importance. It represents the same process that is seen in all cases of arthropathia deformans. The pathologic state of the cartilage must produce an irritation that results in the new formation of osseous tissue. This process is analogous to the formation of callus following a fracture of a bone. The callus with its spongy framework is produced by the periosteum, and its formation is especially abundant if a subperiosteal hemorrhage has occurred. It is therefore not surprising to find a proliferation of connective and osseous tissue follow traumatic injury of the joints. Hemorrhage into the cavities of the joints is also seen in hemophilia and in the attacks of hydrops articulorum hypostrophos. In all these cases, a hyperplasia of connective and osseous tissue is found.

Autopsies in cases of gout of long standing have shown true spur formation, and especially the milder forms of gout demonstrate a clear picture of hypertrophic osteopathy. These milder forms of gout, which are so common in old women in families in which gout is prevalent, are often overlooked and are diagnosed as idiopathic arthropathia deformans. The early history of the patient shows that he has previously suffered from typical attacks of gout. From my investigations, I can say that chronic forms of gout often produce the clinical and roentgen-ray pictures of arthropathia deformans. Of course, not all

cases of simple hypertrophic changes of the epiphysis and of Heberden's nodes are of gouty origin.

The chronic proliferative forms of mild gout represent the transition to the second form of chronic diseases of the joints described by Virchow and Volkmann as arthropathia deformans. These investigators showed that this disease is of a productive and not of an inflammatory character, and that the first changes to be noted are the degenerations of the cartilages. Pommer of Innsbruck and Brogsitter of our clinic have confirmed these observations. All these workers agree that first a calcification and then a new growth of osseous tissue appears whenever a degeneration of cartilage takes place. This osseous hyperplasia occurs particularly on the edges of the cartilage to produce there the well known spurs which lead to a limitation of motion. Often the roentgenogram shows that the production of bony outgrowths is not limited to the region of the damaged cartilage, but extends to the neighboring structures. In such cases it would seem that there must be an irritation with a resulting productive activity in the periosteum. Often this hyperplastic process extends to the synovial membrane and produces polypoid excrescences of a villous character. It must therefore be concluded that the degeneration of the cartilage alone is not a sufficient explanation for the entire hypertrophic process.

Whenever the cartilage is entirely lost, the surfaces of the bones are exposed. Frequently, the cartilage of the opposite surface of the joint is also lost. The bones, therefore, begin to grind off in those places in which they are not protected by cartilage, and marked destruction and deformity of the surfaces result. No signs of inflammation are ever found on the unprotected bony surfaces, and therefore a proliferation of connective tissue never takes place. It is a rule without exception that ankylosis is never found in arthropathia deformans.

In consequence of the absence of inflammatory processes, pain and swelling of the affected joint are not pronounced. The pain that is felt when the patient produces motion in the joint, as in standing or walking, is absent during rest. In the textbooks, advice is given to move the limbs because motion is said to prevent the progressive stiffening. It is questionable whether this treatment is correct. It may be more advisable to protect the joint against excessive movement and pressure, because overactivity on the part of the degenerated joint leads to increased malformation.

Often it is difficult to find the cause for the degeneration of the cartilage. It may be the simple senile atrophy of the cartilage with its subsequent mild bony hypertrophy which has been fully described by Weichselbaum and Pommer. Old age is a progressive and incurable disease, and so also are the senile conditions of the joints. In other

cases, the degeneration of the cartilage is due to mechanical causes. Malpositions of the lower limbs, such as flatfeet, coxa vara and genu valgum, are in later years often associated with arthritis deformans. In a similar manner, the latter may develop following fractures of the joints, old osteomyelites and tuberculosis of the joints.

In many cases, the origin of the arthropathia remains obscure. Chronic arthropathies often occur in the joints of the knees and hips of elderly women, and F. Krause believed that this condition was related to an endocrine disturbance. Assmann strongly objected to this hypothesis. It is to be pointed out that in women many of these chronic degenerations of the joints may appear ten years before or ten years after the menopause. My observations seem to show that the arthropathy in such cases occurs almost always in obese women in whom a large excess of weight must be supported by the old joints in the legs. Favoring this point of view is the fact that a reduction in weight is the best therapy in this form of disease of the joints. I do not wish to deny that endocrine disturbances may produce chronic conditions of the bones. Rothschild and Dewy have described such conditions accompanying pathologic states of the thyroid gland, and not unfrequently I, too, have seen such cases. In these patients the administration of dessicated thyroid gland has produced a good therapeutic effect. The same satisfactory results often follow thyroid treatment in the involvements of the joints that are sometimes combined with scleroderma. The disturbance in the joints in scleroderma sometimes presents the picture of the so-called calcareous gout. In some cases of osteomalacia not only the bones but also the joints are affected, and there can be no doubt that this disease is of endocrine origin.

One of the most typical forms of arthropathia deformans is "malum coxae senile." Here also the disease begins with degeneration of the cartilage. The bone of the caput femoris and the acetabulum are exposed, their surfaces are ground away, and the resulting new formation of bone tissue produces an excessive deformation which extends to the collum and even to the trochanter. In most cases of malum coxae senile, the patient complains of pain only in one side, but when the motility of the opposite hip joint is examined thoroughly, it also is nearly always found to be affected. Often the roentgen-ray studies demonstrate accompanying deformities in the knee, shoulder and fingers. It therefore seems probable that a constitutional and not a mechanical factor alone plays a part in this disease.

Charcot's disease of the joints or "arthropathia tabidorum" also belongs to the category of hypertrophic arthropathies. The involvement of the joints in tabes and syringomyelia shows the same excessive new formation of bone together with a severe destruction of the cartilages.

Sometimes the enlarged knee or ankle joint looks as if it had been shattered to pieces by a severe blow with a hammer. Some neurologists believe that Charcot's disease is due to the degeneration of the trophic nerves to the bones, but I believe that a simpler explanation is possible: the condition of the joints in tabes and syringomyelia is characterized clinically by a complete absence of pain. One may move the severely involved joint in all abnormal directions without producing the slightest pain and even without the patient's knowledge. The absolute loss of sensibility and pain in the joint may lead to overstrain, to damage, and even to fractures, and since the patient is deprived of warnings by pain, he uses and insults his damaged joint so as to make the injury progressively worse.

Assmann believes that the spontaneous fractures of the bones which occur in tabes cannot be explained by the loss of pain alone; rather that they indicate a definite alteration in the bones themselves. If this is true, it must be concluded that the diseases of the joints in tabes are due to a primary involvement of the bones rather than of the cartilages. However, I have never seen atrophic disturbances of the bones in tabes. Indeed, many diseases of the joints have their primary origin in the bones. For example, the involvement of the joints in tuberculosis, osteomyelitis and metastatic tumors is usually secondary to a primary involvement of the bone. I have seen a case of hemolytic icterus in which splenectomy had been practiced with excellent results. After several years, a severe destruction of the hip joint appeared, producing a picture similar to tuberculosis. The microscopic examination of the spleen showed that it was not a simple case of hemolytic icterus or Banti's disease, for the splenic sinusoids were filled with the large cells characteristic of Gaucher's disease. Pick of Berlin has seen similar cases of Gaucher's disease with metastatic processes in the bone producing secondary changes in the joint.

It can therefore be seen that pathologic conditions of many kinds are able to localize in the bones and joints and that one has to take a much broader view of diseases of the joints than before. Microscopic, bacteriologic and clinical investigations must be combined for the further study of this important field.

CONTRACTIONS OF GALLBLADDER SEEN IN MAN

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Broadly, the pitfalls of scientific procedure are fourfold, as is shown when an endeavor is made to answer the following questions: 1. Are all the necessary phenomena accurately observed? 2. Are all the phenomena relevant to the problem under consideration? 3. Are all the phenomena irrelevant to the problem eliminated? 4. Has the actual sequence or interrelation of phenomena been established?

These difficulties are common to both observation and experiment, but each of these methods also has defects and advantages which are peculiar to itself. According to John Stuart Mill[1]:

> The first and most obvious distinction between Observation and Experiment is, that the latter is an immense extension of the former. It not only enables us to produce a much greater number of variations in the circumstances than nature spontaneously offers, but also, in thousands of cases, to produce the precise sort of variation which we are in want of for discovering the law of the phenomenon. . . . . When we can produce a phenomenon artificially we can take it, as it were, home with us, and observe it in the midst of circumstances with which in all other respects, we are accurately acquainted.

In the field of the physiology of the gallbladder, as in all biologic investigation, it is, of course, rarely possible to be accurately acquainted with all the circumstances attending an experiment. There is so little precise knowledge of the effects of anesthetics and surgical procedures on the organism, not to mention the unknown factors, that the conclusions drawn from experiments which depend on these must be taken critically.

Observation, then, has this advantage, that it requires the least tampering with the natural process. Of course, the danger of overlooking some significant circumstance will remain until everything is known, but then what need will there be for investigation? Nevertheless, these two methods are the legs on which science has made every advance. One can only gratefully avail himself of every opportunity for observation and experiment and keep a watchful eye on the dangers that lurk in half knowledge.

Recent progress in the knowledge of the physiology of the gallbladder bears witness to the value of the experimental method in the medical

1. Mill, J. Stuart: Logic, New York, Harper and Brothers, ed. 8, 272.

sciences. The names of Rous, McMaster, Burget, Boyden, Crohn, Winklestein, Aschner, Diamond, Higgins, Mann and Whitaker represent a valiant effort to discover the workings of the gallbladder. However, observation as a method had no place in the investigation of this organ, because it could not be observed under normal conditions. The frequent exposures of the gallbladder by the surgeon did not offer satisfactory opportunities for observation, since a laparotomized man is no more fit to be the object of observation than an experimental animal so treated. Under such conditions man becomes the unintended object of an experiment, and subject to all the new factors, known and unknown, thus introduced. This was recognized by Mann,[2] who said:

> In our experiments as well as in all operations on the biliary tract caution is necessary in drawing conclusions because the possibility of trauma or pressure of a foreign body will greatly disturb the normal action of the gallbladder.

Whitaker,[3] in his experiments on cats, observed that:

> The physical condition of the subject seemed to be an important factor; if he were ill no emptying might occur after feeding, or the attempts were feeble. This would indicate that results obtained by any experimental method in which the condition of the animal was not normal, would be open to question.

It was not until Graham and Cole[4] introduced their method of cholecystography that an opportunity was offered to the clinician, who is essentially an observer, to add to the knowledge of the physiology of this organ. Up to 1924, the normal gallbladder was terra incognita to the clinician. Only when enlarged, thickened, or filled with stones was there any hope of seeing it, and then only in rare and fortunate cases.[5] But Graham and Cole have made it possible to study this organ in its normal state, to note its shape, position, density and emptying time. This is a boon to the clinician interested in his calling and increases the number of investigators of this organ from a few laboratory workers to a host of clinicians and roentgenologists.

Unfortunately, the experimental method, at least as applied to the gallbladder, has as yet not brought forward any conclusive results. This is illustrated by the lack of unanimity among experimenters concerning

2. Mann: A Physiologic Consideration of the Gallbladder, J. A. M. A. **83**:829 (Sept. 13) 1924.

3. Whitaker: Am. J. Physiol. **78**:411, 1926.

4. Graham and Cole: Roentgenologic Examination of the Gallbladder, J. A. M. A. **82**:613 (Feb. 23) 1924.

5. Assmann: Die klinische Röntgendiagnostik der innern Erkrankungen, Leipzig, F. C. W. Vogel, 1924, p. 703.

some of its most vital problems, as shown by their replies to the following questions:

Does the gallbladder contract to expel the bile?

Yes	*No*
Whitaker [3]	Graham [10]
Higgins and Mann [6]	Winklestein and Aschner [11]
Ellman and McMaster [7]	
Chiray and Pavel [8]	
Meltzer [9]	

Does an artificial gallbladder empty itself?

Yes	*No*
Graham [10]	Whitaker [3]

Do respiratory movements influence the emptying of the gallbladder?

Yes	*No*
Winklestein and Aschner [11]	Whitaker [3]
	Higgins and Mann [6]

Does the flow of bile from the liver through the common duct exert a siphonage action on the gallbladder?

Yes	*No*
Graham [10]	Whitaker [3]
	Higgins and Mann [6]

Is the empty gallbladder flaccid or contracted?

Contracted	*Flaccid*
Higgins and Mann [6]	Graham [10]

Do duodenal peristalsis and tonus affect the flow of bile from the gallbladder?

Yes	*No*
Burget [12]	Whitaker [3]
Copher and Kadama [13]	

Does the presence of acid in the stomach increase the flow of bile?

Yes	*No*
Ellman and McMaster [7]	Whitaker [3]
Cole [14]	Bruns [15]

Does the presence of food in the stomach induce the emptying of the gallbladder?

Yes	*No*
Cole [14]	Whitaker [3]

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6. Higgins and Mann: Am. J. Physiol. **78**:339, 1926.
7. Ellman and McMaster: J. Exper. Med. **44**:151, 1926.
8. Chiray and Pavel: Am. J. M. Sc. **172**:11, 1926.
9. Meltzer: Am. J. M. Sc. **153**:469, 1917.
10. Graham: Am. J. M. Sc. **172**:625, 1926.
11. Winkelstein and Aschner: Am. J. M. Sc. **171**:104, 1926.
12. Burget: Am. J. Physiol. **74**:583, 1925.
13. Copher and Kadama: Regulation of Flow of Bile and Pancreatic Juice Into Duodenum, Arch. Int. Med. **38**:647 (Nov.) 1926.
14. Cole: Am. J. Physiol. **72**:39, 1925.
15. Bruns, quoted by Howell: Text-Book of Physiology, Philadelphia, W. B. Saunders & Company, 1922, p. 817.

What is the rôle of the sphincter of Oddi in the emptying of the gallbladder?

Important	*Unimportant*
Meltzer [9]	Whitaker [3]
Chiray and Pavel [8]	Copher and Kadama [13]
Ellman and McMaster [16]	Higgins and Mann [6]
	Graham [10]
	Burget [12]

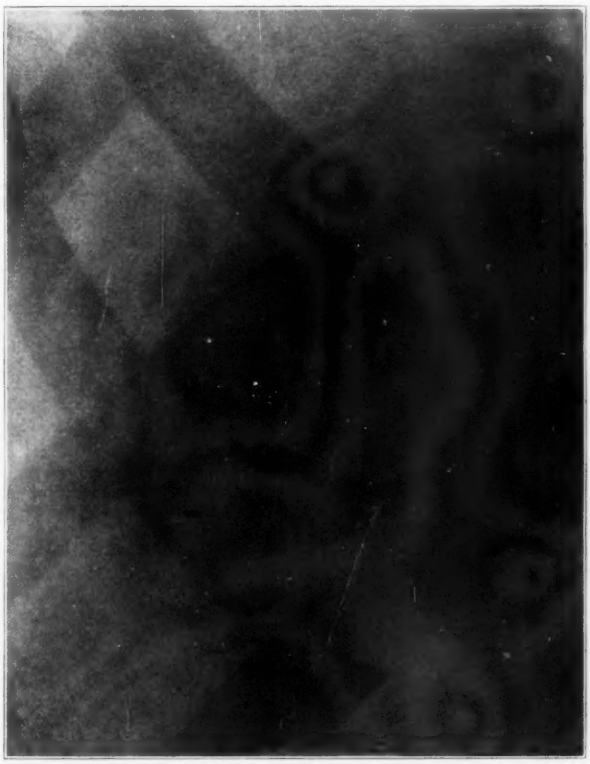

Fig. 1.—Taken before the administration of the dye (tetraiodophenolphthalein). The gallbladder is not visible.

It is evident that additional information is necessary before these problems can be solved satisfactorily. Some of them are beyond the range of the clinician. Others are within his sphere and should be given proper attention.

16. Elhman and McMaster (footnote 7). "We attribute the sudden changes chiefly to the activity of the sphincter of Oddi."

Of the foregoing problems, the most important and the most pressing is the question of whether or not the gallbladder expels the bile by the contractile force of its own musculature. Higgins and Mann [6] have observed localized contractions in the exposed gallbladders of guinea-pigs. Whitaker [3] performed experiments in which iodized oil was introduced into the gallbladder of cats and the abdominal wound was closed. After fasting for twenty-four hours, the animal was given a meal rich in fat, and the gallbladder was examined under the roentgen ray. Marked

Fig. 2.—Figures 2, 3 and 4 were taken fourteen hours after the administration of the dye by mouth. The gallbladder is visible. Its lower pole is oval.

changes were observed in the shape of the gallbladder during the process of emptying.

I studied the gallbladder of a man, a physician, who fourteen hours after the administration of the dye (tetraiodophenolphthalein) by mouth, showed an oval gallbladder (figs. 2, 3 and 4). Two hours after the administration of a meal rich in fat, marked changes were noted in its contour, indicating active contractions in its wall. These changes showed a striking similarity to those in Whitaker's experiment. (Compare figures 5, 6 and 7 with figure 8.) These roentgenograms also

illustrate the observations of Higgins and Mann [6] on the exposed gallbladder of the guinea-pig. I quote from these authors:

The exposed vesicle at the beginning of observation is usually spherical. . . . . Instead of reducing as a unit as a urinary bladder does in expelling its contents, independent centers of contraction induce a variety of patterns in the emptying vesicle. . . . . There are no waves of contraction, extending over the fundus and

Fig. 3.—Taken fourteen hours after the administration of the dye by mouth.

on to the neck of the bladder but only these localized contractile areas. . . . Very frequently we have observed contraction of these regions to form small knoblike diverticula over the entire fundus.

REPORT OF A CASE

History.—L. K., aged 43, a physician, gave a family history which was irrelevant. His wife and two children were alive and well. He drank one cup of coffee a day and an occasional cup of tea, did not use alcohol and smoked

fifteen cigarets a day. He had had the usual diseases of childhood. Up to ten years before presentation, he had had an acute attack of tonsillitis yearly. Since then, he had had only two or three attacks. The tonsils had not been removed. He had not had pneumonia, typhoid, malaria, acute rheumatic fever, any operations or accidents. The present illness began when he was 12 years of age, and since then he had been subject to heart burn. Mucus was present in the stool at the age of 15. This condition lasted five years. When 19 years of age, he first noted a lumpy feeling in the epigastrium and between the scapulae, which lasted

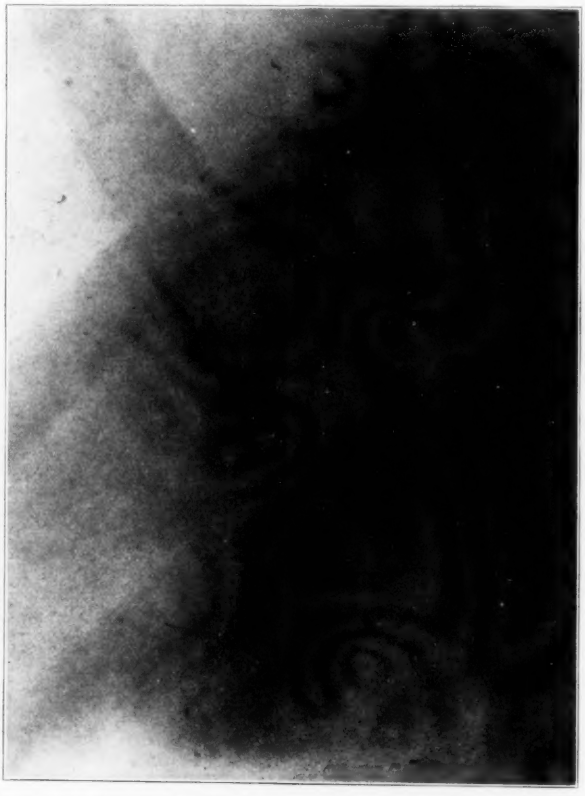

Fig. 4.—Taken fourteen hours after the administration of the dye by mouth.

a few days. He described the sensation as extremely uncomfortable but not exactly painful. The heart burn has continued up to the present, and is not relieved by food; in fact, it is even aggravated by it. Immediate relief is obtained from sodium bicarbonate or magnesia magma.

On Jan. 1, 1918, when the street car on which he was riding made a sharp turn, the patient experienced a sudden, agonizing pain in the epigastrium which radiated straight back. Similar attacks had occurred at intervals varying from once in three days to once in six weeks. They occurred most often two or three

hours after meals, occasionally before meals, and were promptly relieved by alkalis. In fact, the patient had been able to ward off an attack by means of alkalis if taken soon enough. The attacks had lasted from one to forty-five minutes and were not relieved by movement of the bowels or the passage of gas. The pain had awakened him at night only once, five years ago. A sense of pressure in the epigastrium and in the rectum and a desire to urinate accompanied the attacks; the patient belched a good deal after the attack subsided and felt perfectly well within two minutes after it had passed. His appetite was good, his weight was stationary and he had never vomited during or between the attacks. The bowels moved daily, but the movement was constipated. The patient took an occasional mild laxative. About six months before presentation he noticed a few drops of blood on the stool after a large movement.

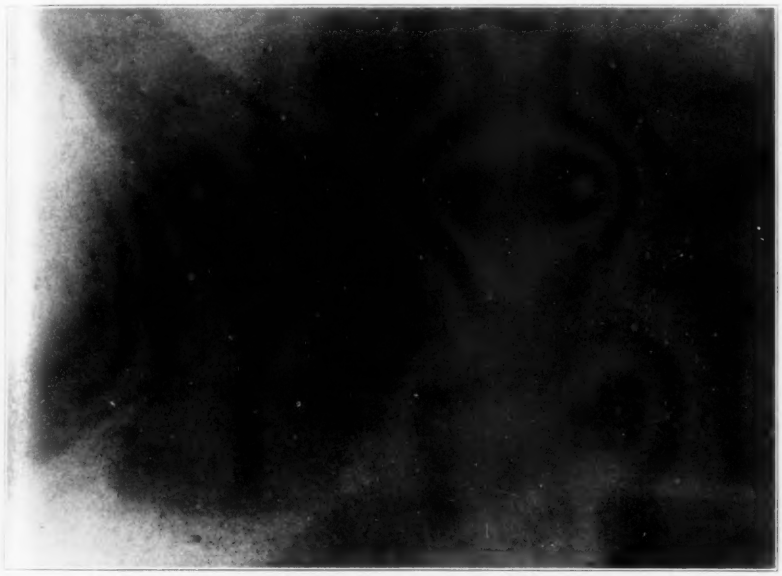

Fig. 5.—Figures 5, 6 and 7 were taken two hours after the administration of a meal rich in fat. The gallbladder is flattened at its base and distended from side to side. At its outer border a protrusion appears in the same location as that seen on the gallbladder of Whitaker's cat. This is most marked on figure 5, less marked on figure 6, and on figure 7, the outer border is almost round.

Nocturia, frequency, dysuria, cough nor dyspnea on exertion had not been noted. The patient has not had headache or dizziness; the vision is fine and he sleeps well. He was never jaundiced.

Examination.—The pulse rate was 72 and regular. The blood pressure was 122 systolic and 82 diastolic on the left arm, and 102 systolic and 86 diastolic on the right arm. The patient weighed 160 pounds (72.6 Kg.). The teeth were in poor condition. The throat was congested, and there was granular pharyngitis. The lungs and the heart did not reveal any pathologic conditions.

The determination of the right Boas point was positive. The liver and spleen were not palpable. The entire right side of the abdomen was tender, but tenderness was most marked in the region of the gallbladder. The gallbladder was not palpable and rocking liver elicited much pain. Pressure over the area of the gallbladder gave the patient the same painful sensation as that experienced during the attacks.

The results of the rectal examination were negative.

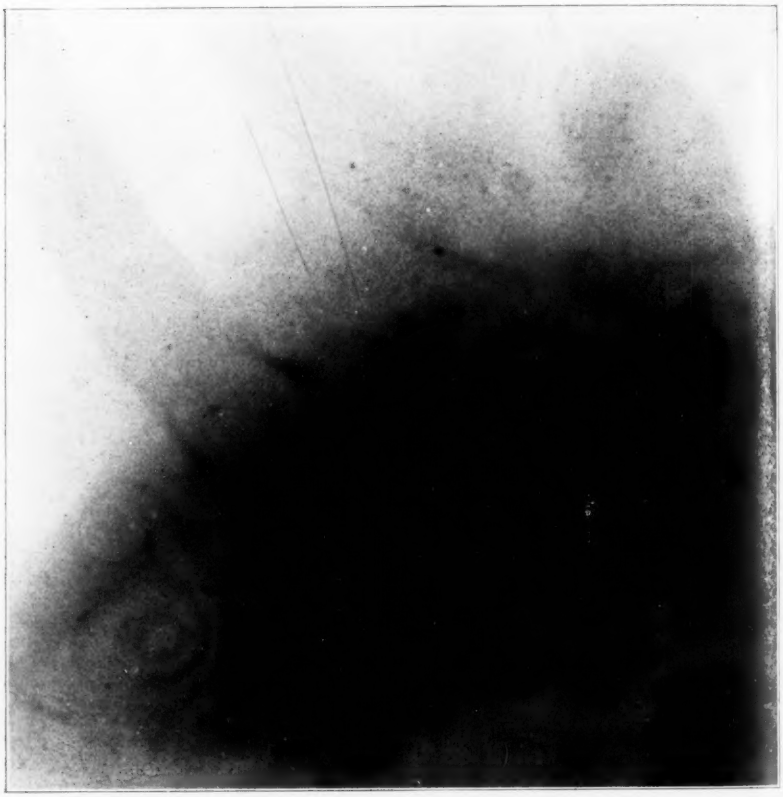

Fig. 6.—Taken two hours after the administration of a meal rich in fat.

An examination of two specimens of the feces revealed an alkaline reaction; the benzidine test for blood was negative; no ova were found. Urinalysis was negative.

Chemical examination of the blood showed sugar, 93 mg.; creatinine, 1.2 mg.; urea nitrogen, 12.5 mg.; icteric index, 8.8; blood bilirubin, $\frac{1}{200,000}$. The blood Wassermann reaction was negative. The blood count revealed: 5,850,000 red blood cells, 76 per cent hemoglobin (Sahli) and 7,400 white blood cells. The differential count showed 59 per cent polymorphonuclear leukocytes, 39 per cent small and

large lymphocytes, 2 per cent eosinophils. An examination of the gastric contents revealed: residue, 12 cc. of clear fluid; free hydrochloric acid, 34; total acidity, 48; negative blood and bile.

A fractional test meal (Rehfuss) was given. The first specimen was removed fifteen minutes after administration of the test meal.

A. M.	cc.	Free Hydrochloric Acid	Total Acidity
1—10:00	20	13	30
2—10:15	22	12	36
3—10:30	20	16	49
4—10:45	20	18	50
5—11:00	40	44	66
6—11:15	20	62	78
7—11:30	20	45	65
8—11:45	20	25	34

A test meal of barium gruel was administered before breakfast. Fluoroscopy of the stomach did not reveal any obstruction in the esophagus. The lowest point of the greater curvature extended two fingerbreadths below the crest of the ilium. The incisura angularis was on a level with the crest. The pylorus did not extend beyond the right border of the spine, was tonic in type and the shape of a fish hook. Peristalsis, time required for emptying, indentations, fluid layer and air bubble were normal. Niches, defects or tenderness were not noted. The duodenal cap was small and triangular, and the apex was directed upward and the base downward. Niches or defects were not seen. Pressure over it did not elicit tenderness, but there was marked tenderness when pressure was applied above it, in the area of the gallbladder. The duodenal loop was not enlarged. Angulations were not present. The foregoing observations were corroborated on the roentgenograms of the stomach and the duodenum.

After four hours, the stomach was empty. The head of the barium meal was in the ascending colon, the remainder in the ileum.

After twenty-four hours, the barium occupied the large intestine from the cecum to the middle of the transverse colon. The appendix was filled and was irregular in outline and beaded. Its direction was upward and to the left, and then transversely to the left.

After forty-eight hours the barium was seen in the large intestine from the hepatic flexure to the pelvic colon. The cecum and the ascending colon were empty, but the appendix was still filled and was irregular and beaded. Its direction was downward and to the left.

After eighty-four hours, only a thin layer of barium could be seen in the distal colon, but the distal portion of the appendix was still filled with barium.

A barium enema was given, the entire large intestine readily filling from the rectum to the cecum. A little barium entered the ileum. The splenic flexure reached up to a little above the lower border of the heart. The hepatic flexure reached up to costal margin. There was considerable spasm in the descending colon and the sigmoid.

Proctoscopy was performed, the proctoscope entering 30 cm. Considerable congestion was noted in the mucous membrane of the sigmoid.

There were no pathologic conditions in the heart or the lungs. Roentgenograms showed the gallbladder clearly fourteen hours after the administration of the dye (tetraiodophenolphthalein) by mouth. Its lower pole was rounded on all three roentgenograms (figs. 2, 3 and 4). Two hours after the administration of a meal rich in fat the gallbladder had hardly diminished in size. Its lower pole was no longer rounded but flattened from above downward, and distended from side to side. A protrusion was clearly seen at its outer border (fig. 5). This assumed a more rounded shape in figures 6 and 7.

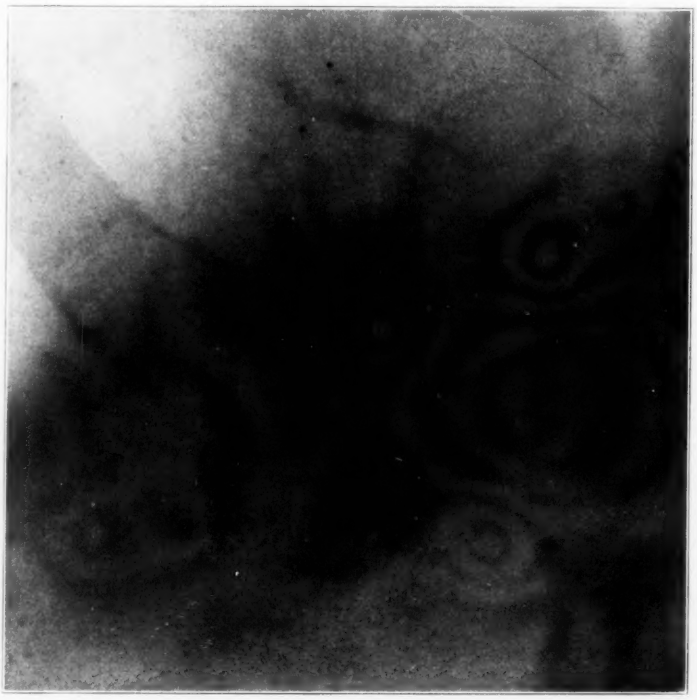

Fig. 7.—Taken two hours after the administration of a meal rich in fat.

COMMENT

Changes in the shape of a hollow organ may be produced in three ways: (1) by pressure or release of pressure from within, (2) by pressure exerted on it from without, and (3) by the contraction of its own walls.

Hydrostatic laws rule out the first possibility (Pascal's principle). Any horizontal layer in the gallbladder has the same pressure of bile at all its points; therefore the release of the pressure should affect all its

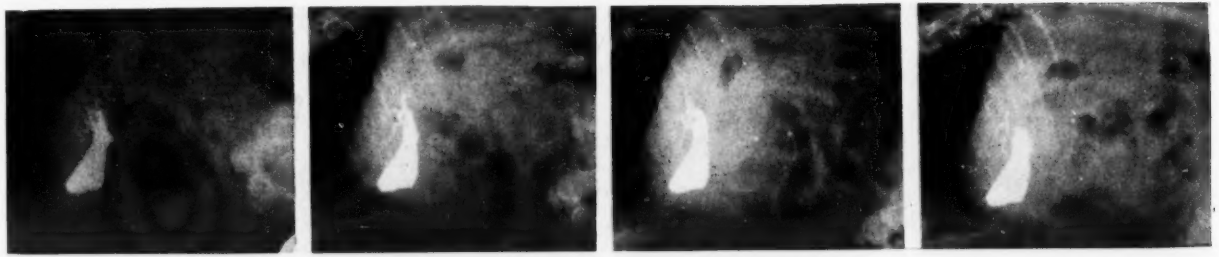

Fig. 8.—Copy of the gallbladder of Whitaker's cat. Compare this with figures 5, 6 and 7, and note the similarity (from Am. J. Physiol. **78**:415, 1926).

parts proportionately. The result should be a uniform collapse of the gallbladder. Instead there are flattened areas in some places and bulging in others.

Winklestein and Aschner [11] offered the second hypothesis. They are of the opinion that the descent of the diaphragm during inspiration exerts sufficient pressure on the gallbladder to "squeeze" the bile into the common duct. From figures 2, 3 and 4, however, it is readily seen that there was no change in the shape of the gallbladder before the administration of the test meal, though obviously respiration was going on at that time. Not until after the administration of a fat meal, which induces the emptying of its contents, were definite changes in its shape observed (figs. 5, 6 and 7). Besides, roentgenograms of the gallbladder are always taken during expiration. The experiments of Whitaker [3] and of Higgins and Mann [6] have, moreover, conclusively disproved this theory.

There remains only the last hypothesis, that the observed changes in the shape of the gallbladder are caused by contraction of its walls. Figures 5, 6 and 7 show flattening of certain areas with compensatory bulgings in others. Changes are observed exactly analogous to the "localized contractions" described by Higgins and Mann in their experiment.

Obviously the gallbladder that I observed was not normal, since it had hardly diminished in size two hours after the fat meal was given. To what extent may these changes in shape be due to its pathologic condition? That these changes were not caused by thickening of its walls or pericholecystic adhesions is evident from the fact that they do not appear in the earlier roentgenograms when the bile was confined within the interior of the gallbladder. They appear only after the meal of fat which induced the flow of bile into the duodenum.

The question arises: Why have not these changes been observed more often? In the first place, roentgenograms after a test meal are taken at a time when the gallbladder is greatly reduced in size, and changes in its contour are not easily detected. This gallbladder, on the contrary, had not diminished in size two hours after the test meal, thus furnishing an opportunity for observing its efforts to empty itself. It is also likely that its abnormal state calls forth an increased effort on its part to expel the contents. A calculus, a stricture or any obstruction at its outlet would accentuate its normal contractions and make them visible. If, on the other hand, contractions did not occur normally, it is hardly likely that it would contract when diseased. However, it must be admitted that since the exact condition of this gallbladder was not known, the reason why the contractions were observed in this case must be largely speculative.

SUMMARY

1. Graham and Cole, by their method of cholecystography, have given clinicians a means of observing the gallbladder without the introduction of new factors which may modify its behavior.

2. The conclusions on the physiology of the gallbladder reached by experiments are open to the objection that abnormal conditions, such as the effects of anesthetics and operative procedures, are introduced.

3. Both observation and experiment are valuable but should be taken in conjunction to supplement and correct each other.

4. A case has been described in which changes strongly suggestive of contractions were observed in the gallbladder of a man two hours after the administration of the fat meal. These changes closely resembled those observed by Whitaker [3] in the gallbladders of cats at the time when they were discharging their contents. They also correspond to the description given by Higgins and Mann [6] of the exposed gallbladders of guinea-pigs while undergoing active contractions.

5. The fact that the gallbladder under observation was apparently pathologic does not seem to vitiate the conclusion that the normal gallbladder undergoes contraction at the time when it empties its contents.

EFFECT OF EXPERIMENTAL PYLORIC STENOSIS ON GASTRIC SECRETION *

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An increase above the normal average in the acidity of the gastric contents is observed in various conditions associated with pyloric obstruction in man. This condition is referred to as "hypersecretion and hyperacidity." In most cases, however, the amount of acidity, although greater than the normal average, is within the limits of normal variation. Therefore, in the absence of a knowledge of the acidity of the gastric contents prior to the onset of the disease causing the pyloric obstruction, it may be argued that the acidity found is normal. Also, since there is usually a gastric retention in pyloric obstruction due to functional or organic disturbances, it is probable that a hypernormal acidity may be due to a retention of a normal amount of gastric secretion, and therefore is only relative. Even if hypernormal secretion did occur, one would not be able to state whether it was due to the obstruction and retention per se or to the disease causing the obstruction.

In considering whether or not pyloric obstruction causes "hypersecretion and hyperacidity," one must distinguish between a relative and an absolute hypernormal secretion and acidity. In relative hypernormal secretion and acidity, the condition would be due to retention of gastric secretion and therefore only apparent; in absolute hypernormal secretion and acidity, the condition would be due to an actual increase in the amount and the acidity of the secretion produced. A gastric analysis in the presence of gastric retention is not an accurate measure of the quantity and quality of the juice secreted. A method is not known whereby one can measure accurately the secretion that is being formed when food is in the stomach. Not even the Pawlow pouch reflects all the secretion that is going on in the stomach during a meal. If the interdigestive or the continuous secretion in patients were observed, which should be done more commonly for scientific purposes, significant figures of hypernormal secretion and hyperacidity might be obtained; but then one would be studying only the intestinal phase of gastric secretion, and the data would not be conclusive unless the interdigestive secretion was studied prior to the onset of the disease.

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Hamburger and Friedman [1] have studied the effect of "uncomplicated" pyloric stenosis experimentally produced in dogs by placing a silk ligature about the pyloric outlet. In order to determine gastric acidity and volume, they caused vomiting by the administration of apomorphine from fifty to sixty minutes after feeding the dogs with a test meal; they then determined the quantity and acidity of the vomitus. They found that moderate stenosis produced little if any change, and that marked stenosis caused retention and hyperacidity; they therefore concluded that the hyperacidity was due to hypersecretion. Such a conclusion is hardly warranted in view of the foregoing facts.

At present, there is no ideal method for the study of the effect of pyloric stenosis on gastric secretion. Production of the Pawlow pouch is the best method to use, but in using the Pawlow pouch, it must be kept in mind that it responds poorly when the stomach proper is distended mechanically,[2] and hence will only partly reflect the mechanical effect of retention by the stomach.

METHODS

A Pawlow pouch was made in twelve dogs by the usual technic. From five to ten control responses were obtained to a standard meal consisting of 200 Gm. of ground meat, 250 cc. of milk and 100 Gm. of bread mixed thoroughly. In the first eight dogs the secretion was collected during two one hour periods prior to the meal, for from three to six hours after the meal and, in a few instances, for twenty-four hours after the meal. As most of the tests made on the first eight dogs were continued for three hours after the meal, the figures given in the tables (tables 1, 2, 3, 4 and 5) are for a three hour period following the meal. In the other four dogs the secretion was collected from six to twenty-four hours after the meal. The results shown in the tables (tables 6 and 7) are for a ten hour period following the meal.

In some cases stenosis was produced by tying a tape a quarter of an inch wide about the pyloric sphincter; in others, by placing an aluminum or silver band a quarter of an inch wide about the pyloric sphincter. Bands of different sizes were prepared in order that they might be accommodated to sphincters of different sizes. The band was placed snugly, but not tightly, about the sphincter. Our desire was to produce a partial stenosis that would lead to a retention of from two to four hours; we were not able to obtain such a result, however.

The degree of stenosis obtained was ascertained by determining fluoroscopically the emptying time before and after the stenosis was made. In the first eight dogs, the exact emptying time after the production of the stenosis was not determined, because the animals had to be transferred some distance before the fluoroscopic examination could be made. To determine whether there was retention, we made a fluoroscopic examination of the animals at the time their stomachs would normally be empty, and expressed the results as "retention,"

1. Hamburger, W. W., and Friedman, J. C.: Contributions to the Experimental Pathology of the Stomach, Arch. Int. Med. **14**:722 (Nov. 15) 1914.

2. Lim, Ivy and McCarthy: Quart. J. Exper. Physiol. **15**:13, 1925.

"marked retention" and "high grade retention," according to the amount of material in the stomach.

For a few days after the stenosis was made, the animal was given only water, meat broth and milk with bread crumbs in small portions until it was found that the standard meal could be ingested without provoking vomiting.

RESULTS

A pyloric stenosis was produced in twelve dogs with a Pawlow pouch. Seven of the twelve lived long enough to give responses to the standard meal; six of the seven were observed for from three and one-half to

TABLE 1.—*Effect of Pyloric Stenosis on Gastric Secretion*
Dog 2* with a Pawlow pouch

Procedure	Amount in Cc.	Free Acidity	Total Acidity	Comment
Control Period				
May 19	19.5	0.41	0.45	
20	28.5	0.45	0.51	
21	17.0	0.37	0.44	
22	12.0	0.40	0.48	
23	19.0	0.41	0.45	
24	16.0	0.39	0.47	
25	16.5	0.35	0.40	
Pyloric stenosis produced with tape, May 25				
May 27	6.0	0.29	0.31	Vomited water but not meat
28	4.0	0.32	0.40	
29	5.0	0.43	0.47	No vomiting
31	7.5	0.14	0.20	
June 2	6.5	0.44	0.51	
3	3.2	0.51	0.56	
4	31.0	0.51	0.58	
5	29.0	0.45	0.51	
7	27.0	0.55	0.59	
8	18.0	0.51	0.56	
July 15	20.0	0.48	0.52	
25	33.0	0.35	0.41	
Aug. 22	37.0	0.41	0.49	
Aug. 22	37.0	0.41	0.49	
8	29.5	0.43	0.48	
10	22.5	0.40	0.45	
12	28.0	0.38	0.42	
15	27.0	0.41	0.45	

* The dog was operated on December 3 to increase the severity of the stenosis. A silver band was placed about the pylorus after removal of the adhesions. The dog died on December 7. The size of the outlet was 8.6 mm. A duodenal ulcer had ruptured into the liver with a resulting abscess of the liver. If hypertrophy of the stomach was present, it was insufficient to attract our attention.

The results are for the three hour period after the meal.

seven and one-half months. Gastric tetany was observed in two of the five dogs that died shortly after the production of the stenosis.

In dog 2 (table 1) a decrease in secretion occurred for ten days after the stenosis was produced; following this period, an increase occurred. The acidity of the secretion was slightly increased, the average total acidity for the control period being 0.46 per cent and for the period of hypernormal secretion, 0.49 per cent. This, we believe, is not a significant increase.

In dog 3 (table 2) a decrease in secretion occurred for seven days after the stenosis was produced; following this period, the amount of secretion returned to normal. In two instances the secretion was slightly

above normal, but the increase was not enough to be significant. The total acidity of the secretion did not show a significant change.

In dog 5 (table 3) a marked hypernormal secretion occurred after the stenosis was produced. The copious secretion made it impossible to prevent digestion of the skin about the pouch.

In dog 6 (table 4) a decrease in secretion occurred for three days after the production of the stenosis; after this period, the secretion was definitely increased above normal. An increase in the acidity of the secretion did not occur.

TABLE 2.—*Effect of Pyloric Stenosis on Gastric Secretion*

Dog 3* with a Pawlow pouch

Procedure	Amount in Cc.	Free Acidity	Total Acidity	Comment
Control Period before Stenosis				
May 18	18.3	0.48	0.53	
19	27.0	0.47	0.53	
20	28.0	0.46	0.52	
21	14.5	0.43	0.48	
22	20.0	0.54	0.63	
23	17.5	0.41	0.47	
24	10.0	0.52	0.61	
25	11.0	0.47	0.52	
Pyloric stenosis made with tape, May 27				
May 28-June 1				
June 1	1.0	0.29	0.36	Vomiting
3	3.2	0.51	0.55	
4	9.0	0.45	0.51	
5	21.0	0.45	0.51	
6	15.0	0.52	0.59	
8	18.0	0.51	0.58	
14	12.4	0.42	0.49	Continuous secretion varied; some days normal, others, three and four times normal
15	21.0	0.43	0.58	
July 26	14.0	0.42	0.52	
Aug. 22	11.0	0.10	0.23	
Nov. 4	11.0	0.21	0.30	
7	34.0	0.43	0.50	
8	20.0	0.42	0.47	
9	33.0	0.41	0.46	
11	28.0	0.41	0.45	
13	24.0	0.40	0.45	
15	21.0	0.36	0.41	

* On November 24, the dog was operated on to increase the severity of the stenosis. A silver band in the form of a circle was placed about the pyloric sphincter after removal of the adhesions. The animal vomited intermittently, and died of distemper on December 7. Roentgen-ray examination revealed a high grade stenosis. Marked hypertrophy of the stomach was not present. The size of the outlet was 8.5 mm.

The results are for the three hour period after the meal.

In dog 7 (table 5) a decrease in the secretion occurred for one week following the making of the stenosis. During the following two months, the secretion remained slightly hypernormal. The secretion was not studied during the fourth and fifth months. During the sixth month following the stenosis, a definite hypernormal secretion occurred. The total acidity of the secretion was not significantly affected.

Dogs 11 and 12 (tables 6 and 7) possessed a refractory pouch, as is demonstrated by the fact that relatively small amounts of their secretion were of low acidity. In both these animals the amount of secretion shown in the tables represents a ten hour collection after the meal. In dog 11, following the stenosis, there was no definite change in the

TABLE 3.—*Effect of Pyloric Stenosis on Gastric Secretion*

Dog 5* with a Pawlow pouch

Procedure	Amount in Cc.	Free Acidity	Total Acidity	Comment
Control Period				
May 26	22.0	0.56	0.60	
27	15.0	0.40	0.51	
28	15.0	0.40	0.51	
29	18.0	0.45	0.54	
31	27.0	0.54	0.63	
June 3	25.0	0.50	0.61	
4	32.0	0.47	0.63	
Pyloric stenosis made with silver band, June 6				
June 7	13.0	0.43	0.54	
8	16.0	0.45	0.54	
14	33.0	0.60	0.68	
15	54.0	0.45	0.63	Continuous secretion increased by three times normal

* The dog was chloroformed and death occurred on June 22. Digestion about pouch due to copious secretion made it impossible to obtain accurate figures. The size of the outlet was 12 mm.

The results are for a three hour period after the meal.

TABLE 4.—*Effect of Pyloric Stenosis on Gastric Secretion*

Dog 6* with a Pawlow pouch

Procedure	Amount in Cc.	Free Acidity	Total Acidity	Comment
Control Period				
May 19	13	0.46	0.54	
20	20	0.46	0.53	
21	22	0.43	0.51	
22	20	0.43	0.52	
23	14	0.43	0.50	
24	19.5	0.46	0.54	
Stenosis made with tape, May 25				
May 27	9	0.30	0.37	
28	9	0.46	0.51	
29	19	0.48	0.55	
31	28	0.49	0.61	
June 2	24	0.45	0.54	
3	25	0.54	0.58	Continuous secretion normal
4	40	0.49	0.59	
8	30	0.46	0.51	
15	27	0.45	0.57	
July 25	25	0.49	0.52	
Aug. 22	22	0.46	0.72	
Nov. 4	27	0.38	0.42	
6	28	0.45	0.53	Emptying time test showed retention
8	39	0.46	0.51	
9	35	0.47	0.51	
12	25	0.44	0.49	
Stenosis made more severe with silver band, stomach was extremely large; a stormy convalescence resulted up to December 15				
Dec. 15	15	0.33	0.44	
19	..	....	....	Vomited meal
30	14	0.30	0.45	
31	39	0.45	0.56	High continuous secretion
Jan. 6	28	0.44	0.49	
10	30	0.36	0.41	Emptying time test showed high grade retention
15	28	0.50	0.52	

* The dog was chloroformed. The size of the outlet was 7.5 mm. in diameter. The stomach was markedly hypertrophied, but not as much as in dog 7. Its length was 19.5 cm. and its width was 14 cm.

The results are for a three hour period after the meal.

TABLE 5.—*Effect of Pyloric Stenosis on Gastric Secretion*

Dog 7* with a Pawlow pouch

Procedure	Amount in Cc.	Free Acidity	Total Acidity	Comment
Control Period				
May 27	15.5	0.35	0.45	
28	27.0	0.45	0.51	
29	36.0	0.51	0.55	
31	39.0	0.52	0.63	
June 2	35.0	0.47	0.56	
3	18.0	0.38	0.50	
4	36.0	0.51	0.56	
Pyloric stenosis made with tape, June 6				
June 6	2.0	....	....	Mucus
7	5.0	0	0.14	
July 14	22.0	0.50	0.58	
15	14.0	0.45	0.58	
25	21.0	0.33	0.41	
Aug. 22	20.0	0.27	0.36	
Nov. 6	38.0	0.42	0.50	
7	43.0	0.44	0.52	
8	33.0	0.46	0.51	Continuous secretion is increased from three to five times
9	52.0	0.46	0.51	
10	55.0	0.45	0.50	
11	48.0	0.41	0.47	
12	38.0	0.41	0.46	
13	37.0	0.41	0.48	

* Stenosis was produced with a silver band on December 4. The stomach was extremely large. A stormy period followed and continued until January 24, when the animal was chloroformed. The dog frequently vomited food eaten twenty-four hours before. Accurate tests could not be obtained because of vomiting. January 10 the following data were obtained: the free acidity for 17 cc. of gastric secretion was 0.11; the total acidity was 0.20. Little secretion occurred from tests on other dates. Several twenty-four hour collections were made, the greatest amount of secretion collected from these tests being 26 cc. Frequently nothing but mucus was collected. The animal was chloroformed, and autopsy performed on January 24 showed a markedly hypertrophied and dilated stomach. Its length was 26 cm., and its width was 20 cm. The size of the pyloric outlet was 4 mm.

The results are for a three hour period after a meal.

TABLE 6.—*Effect of Pyloric Stenosis on Gastric Secretion*

Dog 11* with a Pawlow pouch. Ten Hour Secretion

Procedure	Amount in Cc.	Free Acidity	Total Acidity	Comment
Control Period				
April 6	21.3	40	60	
8	24.4	45	65	
15	33.1	30	40	Emptying time: 4.5 hours
22	25.0	20	32	
May 13	41.3	15	25	
Stenosis made with aluminum band, April 15; a quick recovery resulted				
May 8	20.1	35	47	Emptying time: 4.5 hours
Stenosis increased April 29, first meal of meat given on June 5				
June 5	5.7	32	45	Emptying time: 6.5 hours
16	20.0	10	25	
23	20.0	25	30	
30	11.4	10	22	
July 7	22.8	15	20	
15	26.7	3	10	Mostly mucus
30	40.4	0	8	Mostly mucus
Aug. 6	29.2	27	40	
25	22.9	0	8	Mostly mucus
27	12.9	3	10	Emptying time: 6 hours, mostly mucus
Nov. 3	21.0	0	5	

* The animal was chloroformed on November 15. The stomach was markedly hypertrophied. Acidity is expressed in clinical units.

amount of secretion collected, including mucus; but the actual output of hydrochloric acid was decreased, the secretion after the first month consisting almost entirely of mucus. In dog 12, a decrease in the amount of secretion occurred following the stenosis, but there was no definite change in the acidity of the secretion.

In six of the seven dogs a decrease in the secretion occurred for a few days (from three to ten days after the operation for producing stenosis). Four of the seven dogs (dogs 2, 5, 6 and 7) manifested a hypernormal secretion, one (dog 3) a normal secretion and two (dogs 11 and 12) a hypernormal secretion after the stenosis. Dogs 11 and 12 possibly should not be included, because their pouch was refractory. The acidity of the secretion was not significantly altered.

TABLE 7.—*Effect of Pyloric Stenosis on Gastric Secretion*

Dog 12* with Pawlow pouch. Ten Hour Secretion

Procedure	Amount in Cc.	Free Acidity	Total Acidity	Comment
Control Period				
May 21	21.5	35	50	
28	11.5	35	47	
June 4	13.0	45	55	Emptying time: 5.5 hours
5	18.4	53	70	
11	12.0	7	20	
16	7.7	9	22	
Stenosis made with aluminum band, July 2				
July 16	5.4	17	30	Ate part of meal
30	5.1	3	12	
Aug. 6	19.6	40	55	
20	6.5	22	37	Emptying time: 6.5 hours
25	3.7	20	30	
27	7.9	62	75	
Nov. 3	10.1	32	47	

* The animal was chloroformed on November 15. The stomach was markedly hypertrophied. Acidity is expressed in clinical units.

The continuous secretion collected the twenty-third and twenty-fourth hours after the meal was augmented in three of the four dogs (dogs 5, 6 and 7) that showed a hypersecretion. It was augmented in some tests in dog 3, which showed a normal secretion after the stenosis.

Gastric tetany was observed in only two dogs in which the stenosis was marked (dogs 2 and 10).

The stomach was markedly hypertrophied in four dogs (dogs 6, 7, 11 and 12), the greatest hypertrophy occurring in dog 7. That the stomach was hypertrophied and not dilated was shown by the fact that when it was examined, the mucosa of the stomach was not stretched; numerous rugae were present, and the muscularis was of normal thickness (figs. 1 and 2).

Because dogs 11 and 12 had a refractory pouch, and because the stenosis did not cause augmentation of the secretion, we determined the acidity of the gastric contents. These observations were made after

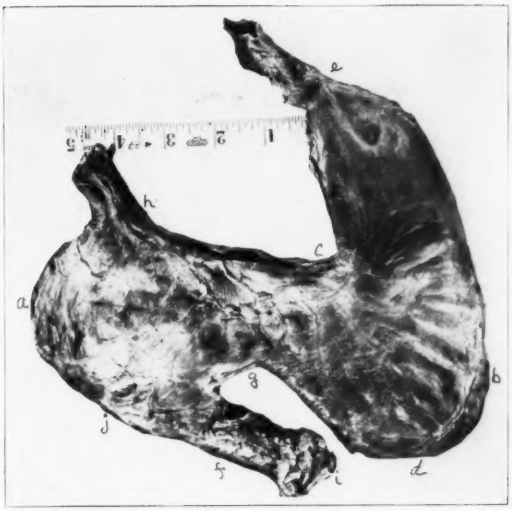

Fig. 1.—A photograph of the hypertrophied stomach of dog 7. The distance from *a* to *b* measures 26 cm.; from *c* to *d*, 13 cm., and from *e* to *d*, 20 cm. The esophageal opening of the stomach is indicated by *h*, and the pyloric outlet by *e* (note the band covered with adhesions). The letter *g* indicates the notch in the fundus produced by the Pawlow pouch; *f*, the pouch; *i*, the roset of the pouch. The normal size of the stomach of a large dog would be represented by the curvature *a j g c h*. Ulcers were not present in the gastric or duodenal mucosa.

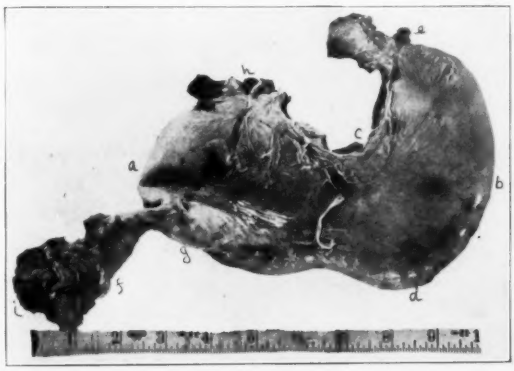

Fig. 2.—A photograph of the hypertrophied stomach of dog 11. The distance from *a* to *b* measures 22 cm.; from *c* to *d*, 9 cm., and from *e* to *d*, 15 cm. The esophageal opening is indicated by *h*; the pyloric opening, by *e*, and the pouch, by *f*. The refractoriness of this pouch may probably be explained by the contraction of the connective tissue which occurred at the isthmus or at the point where the pouch is connected to the stomach proper. In figure 1, it is to be noted that contraction of the connective tissue did not occur.

the stenosis was produced. The reason we did not study the acidity of the gastric contents in all our dogs was that we took it for granted, as claimed by Pawlow and his colleagues, that the pouch correctly reflected the secretion of the stomach as a whole, and that at the time these

TABLE 8.—*Showing That the Pouch Does Not Always Reflect the Secretion of the Stomach; Neither Acidity Nor Quantity is Reflected*

Dog 11* with a Pawlow pouch, August 27

	Secretion of Pouch			Gastric Analysis		
Procedure	Amount in Cc.	Free Acidity	Total Acidity	Free Acidity	Total Acidity	Time, Hour
Continuous meal	2.0	0	3	0	3	11-12
	0.7	0	15	..	...	12-1
	1.2	0	38	0	53	1-2
	2.0	20	45	..	...	2-3
	0.6	5	40	0	68	3-4
	0.5	0	18	..	...	4-5
	0.8	0	3	15	110	5-6
	3.0	0	3	..	...	6-7
	0.7	0	3	28	110	7-8
	1.6	0	3	30	118	8-9
	1.8	0	3	45	138	9-10
	1.7	0	3	..	...	10-11
	1.8	0	3	35	105	11-12
	1.3	0	3	..	...	12-1
	1.9	0	3	20	95	1-2
	1.6	0	3	..	...	2-3
	1.5	0	3	25	88	3-4
	1.7	0	3	..	...	4-5

* Acidity is expressed in clinical units. This is the type of Pawlow pouch preparation that is known in the laboratory as a "poor secreter" and is usually discarded in most studies of gastric secretion. It is used in this study because the pouch is refractory and would be influenced only by maximal stimuli or procedures.

TABLE 9.—*Showing That the Pouch Does Not Always Reflect the Secretion of the Stomach; Acidity is Reflected But Not Quantity*

Dog 12* with a Pawlow pouch., August 27

	Secretion of Pouch			Gastric Analysis		
Procedure	Amount in Cc.	Free Acidity	Total Acidity	Free Acidity	Total Acidity	Time, Hour
Continuous meal	0.5	0	3	0	3	11-12
	0.2	..	..	..	...	12-1
	0.1	0	3	0	40	1-2
	1.2	0	8	..	...	2-3
	0.7	53	78	0	70	3-4
	1.0	80	98	..	...	4-5
	1.2	88	100	0	108	5-6
	1.7	93	103	..	...	6-7
	1.5	90	105	0	133	7-8
	0.8	113	130	..	...	8-9
	0.5	95	120	0	130	9-10
	0.5	75	95	..	...	10-11
	0.5	50	85	..	...	11-12
	0.8	38	50	35	103	12-1
	0.8	35	45	38	100	1-2
	0.4	30	40	..	...	2-3
	0.8	0	3	40	70	3-4
	1.4	0	3	..	...	4-5
	2.0	0	3	..	...	5-6

* Acidity is expressed in clinical units. This is the type of Pawlow pouch preparation that is known in the laboratory as a "poor secreter" and is usually discarded in most studies on gastric secretion. It is used in this study because the pouch is refractory and would be influenced only by maximal stimuli or procedures.

observations were made we did not realize the importance of mechanical distention as a factor in gastric secretion. The results shown in tables 8 and 9 demonstrate, in a striking way, that the pouch does not always

reflect the secretion of the stomach. We cannot state that the stomach proper in these two dogs showed a hypernormal secretion, because we failed to use controls. The acidity of the secretion in these dogs, however, is not abnormally high.

COMMENT

The temporary decrease in gastric secretion following the production of pyloric stenosis is what might be predicted. Dehydration may have been a factor in some cases.

We believe that the results on four of the seven dogs which survived the stenosis reveal that pyloric stenosis may cause a hypernormal secretion, but not acidity. If one rules out the results in dogs 11 and 12, which possibly should be done because in these cases the pouch was refractory and did not reflect the secretion going on in the stomach, our results would show that four of five showed a hypernormal secretion. This observation on dogs with a Pawlow pouch confirms the conclusion arrived at indirectly by Hamburger and Friedman. It also supports the general impression of clinicians that pyloric obstruction is generally associated with a hypernormal secretion.

We believe that the method used in our experiments for the production of stenosis renders only one explanation for the hypernormal secretion possible, namely, that the stenosis caused the increased secretion. However, one can imagine disease conditions in man in which a hypersecretion may be due to the increased absorption of secretagogues in association with a functional pyloric obstruction, for example that which occurs in colitis. One of the authors who has made numerous gastric analyses on himself, and who is subject to intermittent attacks of colitis (irritable colon), has observed that during his attacks of colitis there is a gastric retention, hypernormal acidity of a test meal and an increased continuous secretion.

The explanation of the hypernormal secretion caused by pyloric stenosis is obvious. It is known that the gastric phase of gastric secretion is due to two factors: mechanical distention and chemical contact of substances in food.[2] It is known that hydrolyzed proteins and the products of digestion stimulate gastric secretion from the intestine.[3] It is also known that prolonged contact of digested products in the intestine stimulates gastric secretion,[4] as does acid in the intestine.[5] From these facts it follows that pyloric obstruction may cause hypernormal secretion as follows: 1. Gastric retention prolongs mechanical distention and chemical contact. 2. The more complete the hydrolysis in the stomach,

3. Ivy and Javois: Am. J. Physiol. **71**:583, 591, 604, 1925.

4. Ivy, Lim and McCarthy: Quart. J. Exper. Physiol. **15**:55,1925. Ivy and Javois (footnote 3).

5. Ivy and McIlvain: Am. J. Physiol. **67**:124, 1923.

the greater is the effect of the chyme in the intestine and the more easily are the hydrolytic products in the chyme digested by the pancreatic juice. As a result the intestinal phase of gastric secretion would be augmented. 3. As the chyme is more acid, more acid stimulation of gastric secretion would result and more pancreatic juice would be secreted. 4. The slow ejection of chyme from the stomach would prolong the contact in the intestine. The results of our experiments seem to indicate that the gastric factors are more important than the intestinal factors in causing hypernormal secretion. Another possible factor must be considered, namely, that the stenosis increases the irritability of the local secretory mechanism in the stomach. The hypernormal continuous or interdigestive secretion might be explained either by more prolonged contact in the intestine or by more complete digestion due to more complete hydrolysis in the stomach.

The failure to observe a "hyperacidity" in our experiments confirms the prevalent view held by some physiologists and by some clinicians.[6]

The appearance of "gastric tetany" in two of the animals in which the stenosis was severe was to be expected from the observations of Hamburger and Friedman [1] and others.

The hypertrophy and dilatation of the stomach that we have observed in our animals was also observed by Hamburger and Friedman, who found it two months after the stenosis. The hypertrophy in our animals was especially striking.

The failure of the Pawlow pouch always to reflect the secretory activity of the stomach should be especially emphasized. We have suspected this while working on other problems of gastric secretion, and as a rule have not used a dog with a refractory pouch. Two of these dogs (dogs 11 and 12) were used in these experiments, however, because at the time we were not aware of the importance of the mechanical factor in gastric secretion, and also because we desired to determine whether a hypernormal secretion would occur in "poor secreters," or dogs with refractory pouches. The dictum that the Pawlow pouch in a dog reflects the secretory activity of the stomach cannot be taken for granted as a rule, if for no other reason than that the pouch only partly reflects the mechanical factor. Our results on dogs 11 and 12 show that when one studies the effect of various procedures on gastric secretion, using dogs in which a Pawlow pouch has been made, it must be shown that the pouch at least approximately reflects the secretion of the stomach. This must be done in order to avoid mistakes.

The cause of refractory Pawlow pouches is problematic. One of us (Ivy) explains it as being due to contraction of secondary connective tissue at the isthmus which causes a pressure block of the vagus branches

6. Carlson: Physiol Rev. **3**:1, 1923.

going to the pouch, the blood supply not being interfered with to any extent; in other words, the contraction of connective tissue changes the Pawlow pouch into a Heindenhain pouch, which is known to be more refractory.

SUMMARY

Experimental pyloric obstruction was made in twelve dogs with a Pawlow pouch. Seven of the dogs survived for a period of from three and one-half to seven and one-half months. The stenosis first caused a decrease in gastric secretions; later, four of the seven dogs showed a hypernormal secretion, one a normal secretion and two a decreased gastric secretion. The last two should probably be ruled out because their pouches did not reflect the secretory activity of the stomach. The indirect observation of Hamburger and Friedman is confirmed, namely, that experimental pyloric obstruction causes a hypernormal secretion of gastric juice in some cases. The point is emphasized that before Pawlow pouches are used it should be shown that they reflect the secretory activity of the stomach.

The cause of the hypernormal secretion is discussed.

A marked hypertrophy of the stomach occurred in four of the seven dogs.

THE EFFECTS OF MITRAL AND TRICUSPID INCOMPETENCE ON THE WORK OF THE HEART*

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Previous workers on experimentally induced valvular incompetence in the heart have been almost exclusively occupied with lesions of the mitral valve and their limited aspects, namely, the period of the cycle in which the backflow into the auricle actually occurs, the pressure changes set up in the auricle and ventricle and the factors concerned in the compensation of the defect. Other sides of the problem which are at least as important as those mentioned, have scarcely been considered up to now, such as actual measurement of the output from the heart during the course of a lesion, which is the best criterion of compensation; nor has a contrast of the function with mitral lesions been made with that done in the condition of tricuspid defect. The present communication deals with the results of recent work on these problems and is based on a long series of experiments on dogs, operations having been performed on some 150 animals, with a view of ascertaining the points mentioned. The question is treated as follows:

1. The output from the left ventricle with and without a lesion of the mitral valve is measured, in varying conditions of flow, resistance to ejection, metabolism, etc., while a uniform temperature is maintained for a given series of observations.

2. The output from the left ventricle with a lesion of the tricuspid valve is measured, under similar circumstances to those stated under the first procedure.

3. The significance of certain features of the venous pulse record occurring in the presence of the lesions is considered.

METHOD

The largest number of experiments have been performed with artificial circulation, the defibrinated blood passing through an external system with variable resistance, and then through a spiral tube in a bath for heating to a reservoir for return to the venous and pulmonary side, the whole being a modification of what is called the heart-lung method. A calibrated adjustable tap on the tube from the reservoir gives the rate of inflow, that is, the potential and not necessarily actual inflow, to the superior vena cava and right auricle. The head of pressure in the reservoir may be kept practically constant, when no leak exists in the system, by the addition of a little blood from time to

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time. The output was measured by noting the time necessary to fill a cylinder of 100 cc. capacity at a point in the system beyond the artificial resistance, an equal amount of blood at the proper temperature being at once added to the reservoir so that the head of pressure never varied to more than the extent of 100 cc., a difference in height which causes a negligible variation of supply. This time for 100 cc. is given in seconds in the second column of the tables. The quantity of blood expelled could thus be compared with that permitted to flow in at the venous tap, and the work could be calculated. The tube for the production of the lesions was the same throughout. It was passed into the heart through the appendix and pushed into the ventricle. The lumen of this tube, 4 mm. in diameter, was accurately fitted with a smoothly moving piston; this, on being withdrawn, uncovered a side opening, of similar area to that of the lumen, in that part of the tube which remained in the auricle after the end was pushed down into the ventricle through the auriculoventricular aperture. The valve in systole closed round the part of the tube between the side opening and the lower end, so that regurgitation did not occur unless the piston was withdrawn. This form of auricular tube has already been described by me[1] (fig. 1), a somewhat similar one having been previously described by Wiggers[2] for the ventricle.

The volume of blood regurgitated when the side opening in the auricular portion of tube was free varied with differences of pressures, amount of flow, etc., but it could also be varied to the extent to which the plunger was withdrawn, as the size of the auricular aperture could be altered at will. The area of outlet from the heart to the arterial system varied according to the size of the heart, from about 50 to 120 sq. mm. In some cases a single tube in the innominate artery sufficed; in others two tubes of outlet, one in the innominate and the other in the aorta, were used. The sectional area of the venous cannula of the inlet generally was slightly greater than that of outlet when one tube only was used for the latter, distinctly smaller when two tubes were used for outlet. The recording manometers employed were of the Piper pattern of which the advantages are well known. Special screw adjustments made for me by Boulitte of Paris were fitted to the manometer, so that manipulation was easy after insertion. They were inserted into auricles, large veins and the left subclavian artery in different combinations to give two or three records at a time, the optical system being employed for taking the records.

The manometers were filled with Ringer's solution and all air was expelled from them before insertion. The pressure changes within them

1. Barry, D. T.: J. Physiol. **58:** 362, 1924.
2. Wiggers, C. J., and Feil, H.: Heart **9:**149, 1922.

were photographed by means of light from small mirrors placed on the rubber membranes. The rubber on the manometers was sometimes changed so that the deflections of the beam across the photographic paper varied in extent, but the instruments were always calibrated and the range of the beam was ascertained for every experiment. In some cases it is sufficient to note differences of pressure by a mercurial manometer placed in the arterial system. Electrocardiograms have been combined with many pulse records to verify the phases of the cycle in which

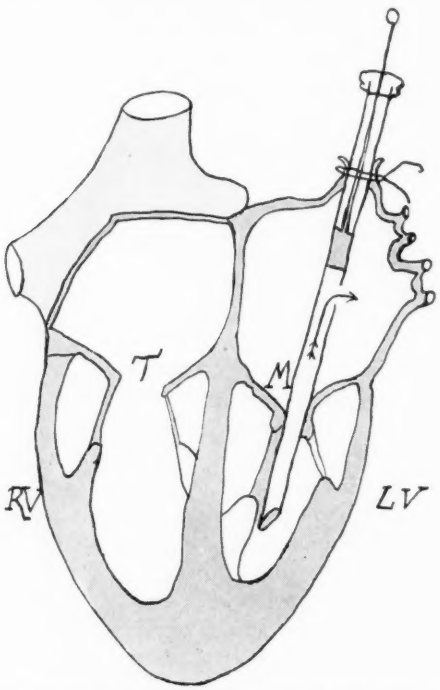

Fig. 1.—Regurgitation tube in position, and mitral leak occurring to the auricle during ventricular systole.

the chief events occurred. Changes in the deflections of the electrocardiogram are caused by valvular defects of the nature of those investigated, but they do not form part of the subject of this work.

The arterial side of the apparatus was tested by means of a pressure bottle giving a maximum pressure of 165 mm. of mercury. With this head of pressure, fluid was freely driven past an artificial resistance of 100 mm. of mercury. Until the difference between the mean arterial pressure and the artificial resistance was reduced to about 20 mm. of mercury a fairly steady flow could be maintained through the resistance.

The artificial resistance was set up by air blown into a glass cylinder through which the blood passed in a soft rubber tube. A manometer connected with the cylinder showed the pressure within it. In actual experiment the balance of pressure on the arterial side of the resistance varied in different conditions. The figures for pressures given on the records were obtained from readings of the mercurial manometer taken during the experiment, and from the range given by the optical manometer in calibration.

The heart with natural circulation has been made use of in some experiments after the injection of heparin or peptone to prevent clotting of the blood. Records of the venous pulse have been thus taken with open and closed thorax, but measurements of output have not been made with natural circulation. Direct measurements of the kind done with the artificial circulation are, of course, out of the question for the natural state with ordinary circulation.

For the insertion of the lesion tube and manometers it was necessary to make small openings in the pericardium, but otherwise this membrane was left intact; in some few cases, however, the pericardium was fully opened, and this is specially mentioned when such cases are referred to in the text. The small incisions made in the pericardium to reach the auricular appendixes do not affect its power of supporting the ventricles and preventing undue distention of them. The influence of the pericardium is chiefly felt in preventing overdistention of the ventricles; when it is cut open over the ventricles in the presence of a lesion and high resistance it rapidly swells to such an extent that the muscle fibers are quickly and permanently damaged by stretching. While the closed sac enhances the power to function, its importance in this class of experiment is also evident in the prevention of the injurious effects of severe tests. When, for instance, the sac is opened and an inflow of 1,200 cc. per minute is permitted, with an artificial resistance of 130 mm. of mercury, a heart weighing 100 Gm. becomes much dilated when valvular defect ensues and does not last long, whereas with intact pericardium it carries on fairly well in these conditions, although the full potential flow is not ejected.

The thorax also influences the function of the heart. This is well recognized, and Howell [3] even says that investigation of output when the organ is exposed does not give reliable results. That is true for conditions with natural circulation, but it is difficult to imagine how the open thorax could so affect the value of results with the heart-lung method.

The influence exerted by the natural respiratory movements is certainly annulled when the chest is opened, and this hampers action

3. Howell, W. H.: Text Book of Physiology, Philadelphia, W. B. Saunders Company, 1927.

when one is working with natural circulation. There are further objections to the method with natural circulation and open chest walls for the present purpose, namely, the difficulty of measurement and control of flow and resistance. But all such objections are overruled with the heart-lung preparation, in which the venous supply is independent of respiratory aids. It may be thought that the artificial inflation of the lungs necessary with the open thorax causes an obstruction to the pulmonary circulation. I have frequently caused free expansion of the lungs without altering the output. Similar observations have also been made by Romm.[4] Accordingly, there is no reasonable objection on such grounds to basing conclusions concerning the function of the heart on observations made in these conditions, and this especially applies in the determination of differences in work caused by lesions, etc., rather than of what a given organ is capable of doing in a general way.

Three chief sets of conditions have been chosen to work with for the preparation. First, the artificial resistance was kept at about 70 mm. of mercury, though this was sometimes raised, and the venous flow was not greater than 700 cc. a minute. These and lower figures represented best the conditions in which output corresponded with potential inflow when one arterial cannula was used for output. With higher flow and pressure, some discrepancy was noticed between output and supply of blood. In the second series, an aortic cannula for outlet was added to that in the innominate artery. The artificial resistance was kept at 120 mm. or lower, and the inflow per minute was from 800 to 1,000 cc. The third group comprised those experiments in which a resistance up to 150 mm. of mercury, and a flow reaching to as much as 1,500 cc. per minute, were employed. More severe tests than these were not considered of practical utility. The vagi were cut before the artificial circulation was started. Patterson and Starling stated that a heart weighing 56 Gm. can deliver 3,000 cc. of blood per minute.[5] My own experience is that in such conditions the heart deteriorates quickly, if valvular defect is occasionally introduced, and that it is not advisable to exceed a flow of 1,800 cc. for any length of time when one is dealing with experimental lesions. This aspect of the question will be referred to again. It is necessary to state that the insertion of the lesion tube does not affect the capacity of the organ to function.

The hearts used were large and the weights given seem high. Stripped of the large vessels, pericardium, etc., the organs generally were about 0.6 to 0.8 per cent of the body weight.

4. Romm, I. O.: Arch. f. d. ges. Physiol. **203**:113, 1924.
5. Patterson, S. W., and Starling, E. H.: J. Physiol. **48**:357, 1914.

SECTION 1

In the comparison of mitral and tricuspid defects, it is essential to bear in mind one important and constant difference between them in the condition of the pulmonary circulation. It has been pointed out that obstruction of the pulmonary circulation accruing from mitral lesions aggravates pulmonary edema and hastens its onset when the state of the blood is favorable to its occurrence. In a right side lesion the aerating surface of the lungs is not affected and the oxygenation of the blood is carried on efficiently, whereas the pulmonary congestion resulting from uncompensated mitral trouble soon becomes an effective cause of anoxemia; so that apart from the mechanical disability, which is greater with mitral interference, the loss in aeration intensifies the effect of this latter lesion. This difference has been remarked by Doumer[6] in the effects of cardiac thrombosis on the right and the left side when the heart muscle is not directly impaired. In chronic lesions one can distinguish between the right and the left side in the preponderance of hepatic or pulmonary symptoms, respectively. The point will be considered again in section *2*.

The extent to which output is affected by defective closure of the mitral valve depends, apart from the magnitude of the defect in itself (size of leak), on three main factors; the degree of resistance on the arterial side, the amount of venous flow and the state of metabolism of the organ. Of course, the weight of the organ used must be taken into account with uniform dimensions of leak. The two first conditions are an expression of the amount of work to be performed, and of these the resistance is the more important, while the third factor is concerned with the state of the muscle which has to effect it. As a general observation it may be stated that with an artificial resistance not exceeding 100 mm. of mercury and a venous flow of 1,000 cc., a heart weighing 100 Gm. is definitely affected in efficiency by a mitral lesion of the dimensions employed in this work. It is not easily compensated, and though the output rises in the course of four or five abnormal beats it does not reach the quantity ejected prior to the lesion until the leak is closed or the artificial resistance much reduced. The balance of pressure on the arterial side necessary for forcing of the resistance is not recovered otherwise. It is important to note that mere increase of venous flow, though it leads to some increase of output, is not sufficient to restore the previous output. That the presence of the tube is not the cause of defective compensation is implied in what has already been stated, that the insertion of the tube does not per se diminish output; there is an immediate return of efficiency when the piston is pushed down, as is obvious from the records (figs. *2* to 6). The record in figure *2* is that of a heart of

6. Doumer, E.: Echo méd. du nord **29**: 243, 1925.

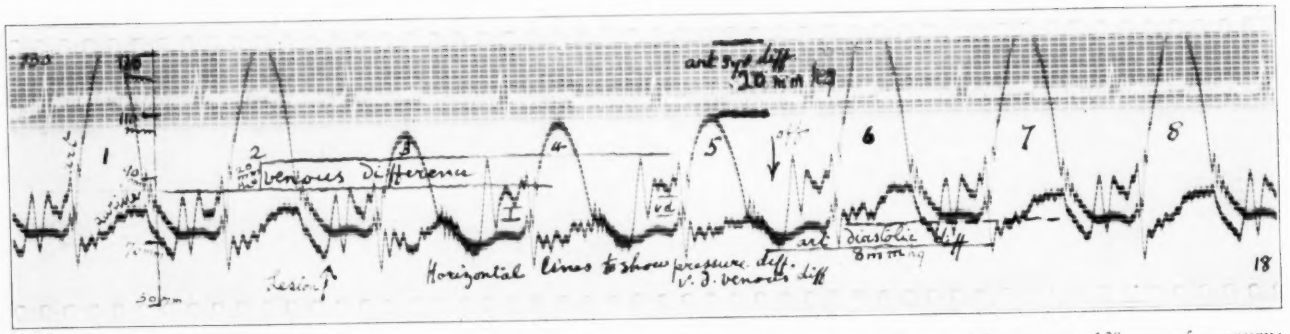

Fig. 2.—Records from left subclavian artery, and right auricle; mitral regurgitation; systolic arterial pressure—130 mm. of mercury; diastolic arterial pressure—75 mm. of mercury; lesion at arrow after second cycle; nos. 3, 4 and 5 are regurgitant cycles; fall of systolic pressure equal to 24 mm. of mercury in cycle 3; fall of systolic pressure equal to 22 mm. of mercury in cycle 5; fall of diastolic pressure equal to 8 mm. of mercury; instant recovery of arterial pressure when the leak was shut down at inverted arrow after cycle 5; further description is given in text; the time in all records except 1 and 2 is given at the top in tenths and twentieths of a second.

115 Gm. with a venous flow of 1,000 cc. and artificial resistance of 100 mm. of mercury. The manometers were inserted into the right auricular appendix and left subclavian artery. The fall of systolic arterial pressure due to lesion is well demonstrated (about 20 to 22 mm. of mercury). In a later part of the record, with repetition of the lesion, this lowered pressure was seen not to rise more than from 6 to 8 mm. of mercury after half a minute's duration of defect in the valve. The record was taken within a quarter of an hour from the beginning of the artificial circulation, and nearly all the results given were obtained from organs not later than half an hour from this starting point. Experience has shown that a preparation begins to deteriorate after this interval. This is especially true if more than a thin ring of tissue is tied off from the auricles when the tube or manometers are inserted. A small portion of this tissue is sufficient to prevent bleeding at the site of the ligature. It has also been noticed that prolonged high resistance to output has a depressing effect. If after a lesion has existed for some time the output and arterial pressure are quickly restored to their former level when the leak is stopped, as happened in several trials with the organ from which figure 3 was taken, it is concluded that the heart has not been affected by the abnormal conditions, and it may be employed for further tests.

It is impossible to state the actual amount of reflux in each case, but it has been calculated to vary from 2 to 7 cc. per beat, according to relative pressures in aorta and auricle. The aperture for leak was relatively large compared with the sectional area of the aperture of outlet when this was confined to the innominate artery, because similar relative dimensions must be common in natural defects. Wiggers [2] has shown that in the heart with natural circulation compensation of a small leak is fairly good.

With regard to the condition of the heart muscle, it can be easily affected by an alteration of the amount of ventilation of the lungs, and this is best done by means of an aperture in the tracheal cannula, the size of which can be varied at will. The opening in the cannula was uncovered for defective aeration. When the supply of air is diminished in the presence of a lesion, the function of the heart usually drops in quantity; the organ fails to eject an amount of blood equal to that previously expelled. When a lesion is not present, the same degree of anoxemia, in otherwise similar conditions, does not cause an equivalent reduction of output. This has been observed frequently when in given circumstances the output was reduced by the setting up of a valvular lesion and was still further diminished when the air supply was cut down. Less than a minute was required to bring about the deficiency of function caused by limiting the expansion of the lungs.

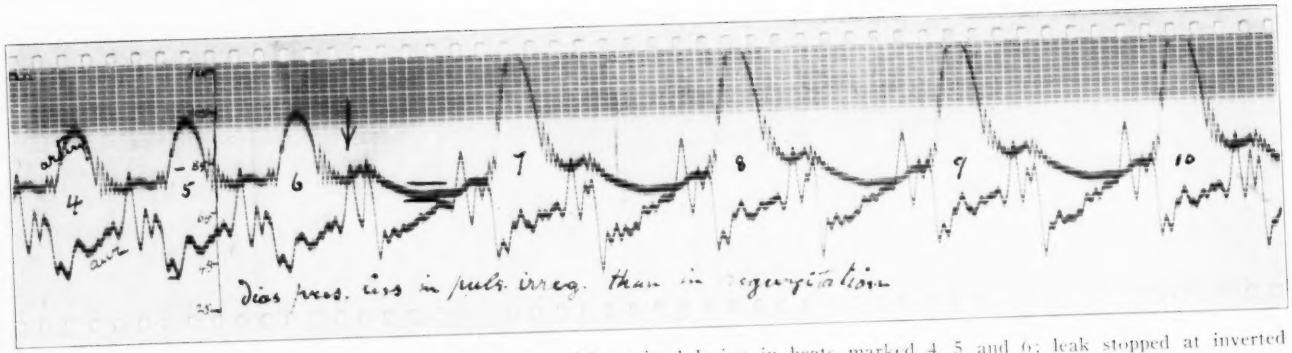

Fig. 3.—Records from subclavian artery and right auricle; mitral lesion in beats marked 4, 5 and 6; leak stopped at inverted arrow; the succeeding beats show arrhythmia with a rise in auricle pressure.

After-effects of lesions are sometimes observed, and they take different forms. A continued diminution of output and failure to recover the initial arterial pressure are of common occurrence. Extrasystoles frequently appear during and after the lesion, and arrhythmia is sometimes established (figs. 3 and 4). The record in figure 3 was taken to show the abolition of a lesion which had existed during six cycles. The ensuing abnormal rhythm continued for a period of twenty-one cycles, and the returning normal beat (twenty-second cycle from shutting down the leak at the arrow in figure 3) is shown in figure 4.

The figures in table 1 give some idea of the interference with the circulation caused by mitral leak with constant average ventilation. It shows too that the output is sometimes deficient with respect to supply, but this may be due to slight errors in calibration, even though the latter is effected under uniform conditions of temperature and viscosity of the fluid used.

TABLE 1.—*Results with Hearts Weighing 100 Gm.*

Minute Volume Inflow, Cc.	Time in Seconds for Output of 100 Cc.	Minute Volume Output, Cc.	Difference, Cc.	Rate	Difference per Beat, Cc.	Artificial Resistance	Temperature, C.	Lesion
800	8	750	50	80	0.625	90	37	0
800	11	545	255	70	3.6	90	37	+
800	8.6	700	100	80	1.25	120	37	0
800	13	461	339	70	4.8	120	37	+

The time of output of 100 cc. is given in table 1 for different conditions, and the minute volume calculated from this may be contrasted with that of potential inflow. The absence and presence of lesions are indicated by the signs 0 and + respectively.

The normal state of the heart is not necessarily restored immediately after the defect is remedied, nor is its working power constant at different periods during the influence of the defect, although the conditions of ventilation, pressure, etc., remain the same. Ordinarily, however, it is remarkable to note the consistency of the results. The estimates remain the same in numerous trials for given conditions over long periods, and the time of output is repeated with considerable regularity. It is sometimes difficult to make sure that a lesion is producing its full effect, the intensity of the thrill felt being deceptive according to the direction of the jet. If the opening of the tube within the auricle is too high, the walls of the appendix may be drawn tightly over it and oppose much resistance to the occurrence of a leak. When contradictory or anomalous results are yielded with a lesion, some such cause will generally be found, and it is then necessary to shift the position of the tube or merely to rotate it. One must also avoid pushing the tube too far down into the ventricle. In a small

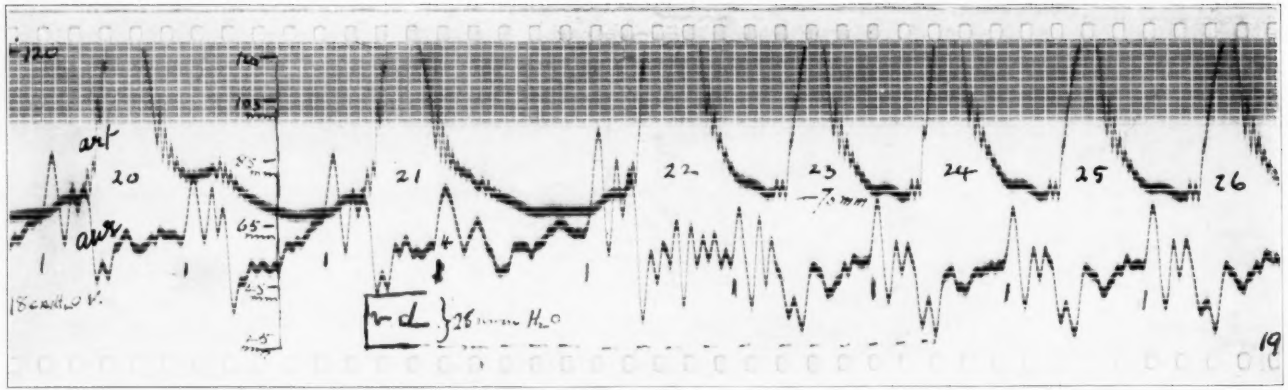

Fig. 4.—Continuation of figure 3 with cessation of arrhythmism in twenty-second ventricular cycle after mitral lesion; the auricle cycle marked 4 is premature, and this heralds normal rhythm, with marked fall in venous pressure.

heart, a second opening on the wall of the tube, a few millimeters from the lower end, obviates the possibility of blocking this end when it touches the ventricular wall. The same tube was used throughout these experiments and was found suitable for every size of heart employed. Once a preparation has been made, the greatest care should be taken to ascertain, by withdrawal of the plunger, that a full leak has been established; then, with manometers properly fixed in position, nothing need be disturbed by the subsequent manipulations and the preparation is reliable.

As showing the regularity with which counts are repeated for the same conditions, an example may be cited from an experiment with a heart weighing 90 Gm. With the inflow tap marking 700 cc. and an artificial resistance of 70 mm. of mercury the time of output of 100 cc. was 8.6 seconds without lesion and 10 seconds with lesion. These times never varied by more than 0.2 second for like conditions in the course of four estimates spread over half an hour, various counts under other conditions having been made in the intervals. Differences become more marked as the work called for increases, according to the size of the heart. With a large heart giving an output of from 15 to 20 cc. per beat or more, a lesion caused by the tube used is almost fully compensated after a few cycles, when the artificial resistance is not too high. In an animal weighing 20 Kg. the heart weighed 120 Gm. In experiment this heart gave with a flow of 1,300 cc. a count of 4.6 seconds without lesion, and 5 seconds with lesion for an output of 100 cc., with an artificial resistance of 100 mm. of mercury. That represents, with a rate of 90, a deficit caused by the lesion of little more than 1 cc. per beat. When the resistance was increased to 140 mm. of mercury, the counts were 6 seconds without and 8 seconds with lesion. The fall in systolic arterial pressure following the lesion was small in the first instance, but it amounted to 15 mm. of mercury in the second. As long as the resistance remained at about 100 mm., little diminution of function resulted from the defect. Of course, there is a liability to error in counting, but in carefully repeated observations it does not amount to more than + or —0.2 second.

The influence of an increase in the peripheral obstruction in its effect on the compensatory reaction of healthy muscle is significant. So far as these artificial conditions indicate, the obstruction is a strong factor in reducing efficiency as measured by the work done with lesion.

The lowering of diastolic arterial pressure resulting from lesion has been considered an aid to ejection. In this connection it is necessary to consider a few points which have presented themselves in the present work. The diastolic fall is shown to vary from a few to about 22 millimeters of mercury in an average case, while the peripheral resistance remains fixed. In the natural vascular system the mean pressure

gradient is fairly uniform at different distances from the heart to the capillaries. In the artificial scheme one can scarcely speak of a pressure gradient between the heart and resistance tube. The obstruction at this latter point is abrupt. When the pressure is lowered on the proximal side of this obstruction, there is greater facility of output into the arterial system, but no reduction of the resistance to onward flow at the tube. A due circulation past this obstruction depends on an ample mean pressure in the arterial system. The lowering of diastolic pressure due to lesion, while it facilitates systolic output, diminishes the available power to force the peripheral obstruction. When the difference between the arterial pressure and the fixed resistance was reduced to about 20 mm. of mercury, the flow to the venous side was greatly diminished, and intermittence occurred. Hence, in the artificial scheme the arterial system is placed between two difficulties, one at the cardiac end demanding a reduced pressure and one at the peripheral end demanding a high pressure. Any increase in this peripheral resis-

TABLE 2.—*Results with Hearts Weighing 120 Gm.*

Minute Volume Inflow, Cc.	Time in Seconds for Output of 100 Cc.	Minute Volume Output, Cc.	Difference, Cc.	Rate	Difference per Beat, Cc.	Artificial Resistance	Temperature, C.	Lesion
1,200	5	1,200	—	90	—	120	36	0
1,200	5.4	1,110	90	90	1	120	36	+
1,200	6	1,000	200	90	2.2	140	36	0
1,200	7	857	343	80	4.3	140	36	+
1,200	9	666	534	80	6.6	150	36	+

tance has always a marked effect in increasing the harm caused by lesion. In natural conditions, when the arterioles are rigid, a comparison may be made with the artificial scheme which provides an indication for relief of the obstruction in conditions of the heart. In table 2 will be seen some details of the effects of a lesion in a large heart with the introduction of high resistances and large venous flow. One is led to think that in natural lesions of the valve in man the peripheral resistance in the arterioles, as compared with arterial resistance, that in a finger contrasted to that in the arm, for instance, is an important condition to determine. A high resistance in the arterioles may coexist with a comparatively low mean or diastolic arterial pressure. The pulse pressure is reduced, and the diastolic pressure remains high.

In the cases recorded in table 2 the valvular defect at first caused moderate reduction of the volume of output amounting to about 1 cc. per beat. In calculating the work the total resistance was arrived at by adding from 25 to 35 mm. of mercury to the fixed resistance to allow for the excess of mean arterial pressure over this. The actual function has been calculated for most of the observations. For the preparation in table 1 it was estimated as follows: With an output of

750 cc. and a rate of 80 (read on the record), the output per beat was 9.4 cc. This was driven against a total resistance of 115 mm. of mercury (90 + 25). So with a sectional area of outlet of 50 sq. mm., the work per beat was about 1,400 Gm. cm. or 112,000 Gm. cm. for a minute's function.

When the lesion set in, the output per beat was $\frac{545}{70} = 7.7$ cc. This was ejected against a resistance of 100 mm. of mercury (the mean arterial pressure had fallen about 15 mm. of mercury), and the work accordingly amounted to about 1,150 Gm. cm. per beat, a reduction of 325 Gm. cm., or 24,500 Gm. cm. per minute. The output per beat varied in ordinary conditions from 9 to 20 cc. The change of rate resulting from a defect is slight, as a rule, unless some form of arrhythmia sets in. That mentioned in the foregoing calculation for Table 1 was exceptional. In figure 2, for instance, there is practically no change of rate, while in other cases it amounts to a few beats. Curves have been plotted at different intervals after the onset of a lesion to show the relation between output and resistance when the flow, temperature, etc., are constant. They show somewhat irregular form beyond a certain pressure which varies for different preparations.

Even when valvular defects are not present the function of the heart deteriorates with anoxemia; but the deficit is greater and more quickly established when the lesion exists. An increase in the inflation of the lungs in the presence of a lesion will cause a marked improvement in the output when this has been lowered by deflation.

Another condition which should be briefly considered under this section, is that of the heart stripped of its pericardium. The deficiency of compensation, compared with intact condition, becomes apparent only with high resistance. A lesion set up in such conditions causes the heart to become distended and the output to be greatly reduced. The minute volume of output may be reduced by an increase of resistance in the normal condition, when the pericardium is opened; and when the lesion is introduced the effect of increased artificial resistance becomes marked, the function being cut down to small proportions. Apparently, a mitral lesion in certain circumstances causes some disturbance of the heart's efficiency, which is not simply measured by the withdrawal in reflux of a certain quantity of blood from the volume to be ejected. Probably a lowering of coronary pressure affects it. Cardiometer records are not of much use for showing the increase of systolic volume when the pericardium is open, because the distention is to some extent masked by the instrument. Without the pericardium, the leak alone may be less than the measure of failure in output following the lesion. The heart in these cases remains distended in systole out of proportion to the diastolic volume, and its power of contraction

is definitely interfered with, an interference which must be independent of anoxemia, because of its rapid appearance.

To conclude this section it is necessary to allude to previous work on the measurement of output from the heart when no lesion existed.[5] For instance, it was said that the maximum of output may be 3 liters for a heart weighing 56 Gm. The hearts used in the present experiments were in general much larger than this; it is necessary to employ large organs to produce lesions easily. The artificial resistance is not stated in the report mentioned. Presumably, the output was effected through two cannulas, in the innominate artery and the aorta, but measurement of the exact amount of inflow is not given. As already stated, I was not concerned with these large quantities of blood for output, but it was found that for the output of 2,000 cc. per minute, the artificial resistance had to be comparatively low. Contrary to what might be expected from the results of the observers here referred to, although they were not concerned with valvular defect, the artificial resistance is an all important factor in aggravating the effects of lesions. In the presence of a mitral lesion in practically any circumstances, an increase of the fixed resistance almost invariably leads to a further reduction of output which may be slight but is generally noticeable or considerable.

SECTION 2

It has been said by Mackenzie[7] that tricuspid regurgitation is common in "normal" human beings, and that it may be in part responsible for the formation of the *V* wave in the venous pulse. The force exerted in systole of the right ventricle is somewhat less than half that of the left, but the difference of resistance to be overcome corresponds pretty well with this. It is important to consider a comparison of the differences prevailing between aortic and left auricular pressures in dealing with tricuspid and mitral lesions, respectively. Obviously, the difference favors a much larger jet on the left side than on the right side, if one may judge by comparisons of the corresponding records (fig. 5 with fig. 6). The same manometer was used for both sets of records, but in different hearts. The conditions of pressure, flow, etc., were the same, and the hearts corresponded closely in weight.

Near the center of the summit of the reflux wave in the right auricle (tricuspid lesion) are seen two or three large sharp oscillations which correspond in time with the vibrations due to the semilunar valves on the arterial curve. One of these oscillations marks the beginning of diastole, generally the highest; it is like the pointed elevation described by me[8] on the *V* wave in man; it marks the highest point of intra-

7. Mackenzie, J.: Am. J. Med. **134**:12, 1907.

8. Barry, D. T.: J. Physiol. **59**: 293, 1924.

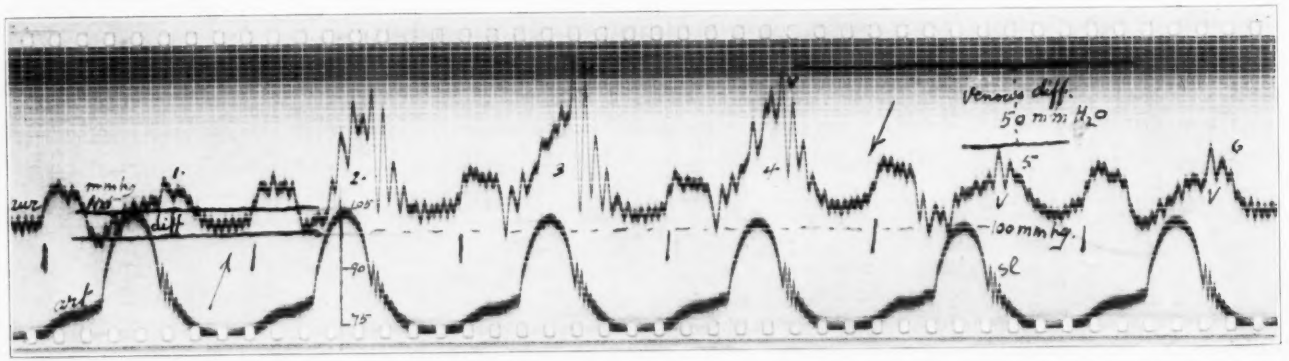

Fig. 5.—Left subclavian artery and right auricle; tricuspid lesion; well marked effect of reflux in beats 2, 3 and 4; defect produced at upward arrow, and abolished at downward arrow.

auricular pressure with tricuspid defect, but not necessarily with normal valve. It is fairly distinct from neighboring oscillations and is seen here even with open thorax, though in the venous pulse generally it is well marked only when the chest is closed. It has, in fact, been ascribed to a possible intrathoracic pressure change, though its causation may be the closure of the semilunar valves. It will be referred to again in section 3 and is mentioned here as marking the summit of the reflux curve.

Regurgitation from the right ventricle has been occasioned by the same tube with a method of introduction through the right auricular appendix similar to that used on the left side. It is evident from the records that output from the left side of the heart is not affected by tricuspid defect in the same way as by mitral defect. The resulting fall of systolic arterial pressure is slight and gradual with the former, measuring no more than from 6 to 8 mm. of mercury in the course of several beats. The returning beat, after the lesion is abolished, usually shows a continued fall, never a rise, of pressure in the arterial system (fig. 5).

The effect of tricuspid incompetence is well marked on the venous side, but the pressure in the right auricle does not rise to a height equivalent to that caused on the left side by mitral trouble, nor does it affect the onward propulsion of the blood to any like extent. The tidal flow after reflux provides a sufficiently large volume for output to keep the pressure in the pulmonary artery nearly up to its former level; and a small increase in inflow is sufficient to restore the arterial pressure (systemic) to its former level. An increase in inflow cannot effect a similar degree of compensation with a mitral lesion, the essential change in the latter condition being a reduction of resistance. Consequently, primary lesions of the tricuspid valve are of less importance than left side lesions from the point of view of function. Increase of artificial resistance has not, in the case of tricuspid leak, any greater effect on arterial flow than such increase produces when no lesion exists; for instance, a preparation in which the heart weighed 100 Gm. was yielding an output of 850 cc. with an inflow of 900 cc., against an artificial resistance of 120 mm. of mercury. A tricuspid lesion was then set up, and the time of output for 100 cc. was 7.4 seconds, the previous time being 7 seconds. On turning the inlet tap to near the mark 1,000, the time for filling the 100 cc. cylinder was reduced to 6.6 seconds, even in the presence of the lesion. Many estimates have been made in this way for comparison with left side lesion effects, and all point to the conclusion stated previously.

Defects in pulmonary ventilation, too, have less effect with right than with left side interference. Defective oxygenation affects the muscular work independently of lesion, the ill effects becoming more

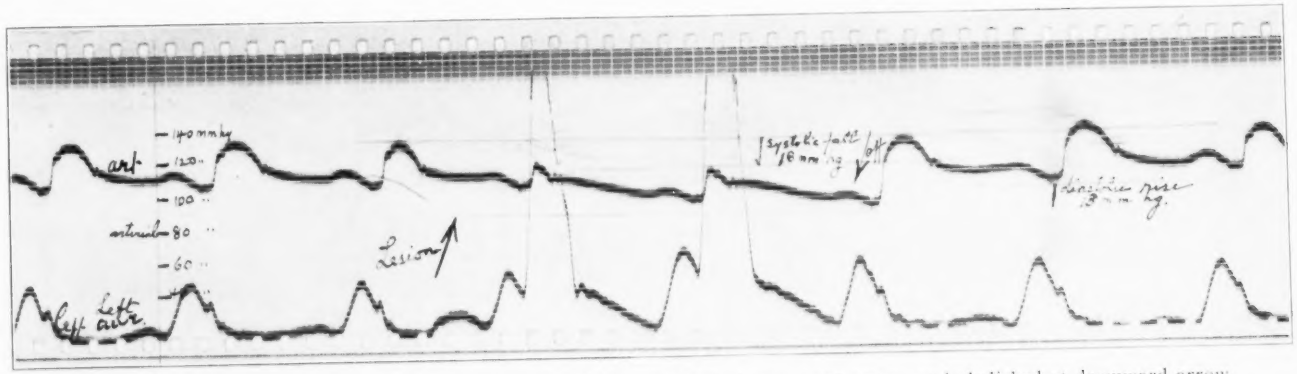

Fig. 6.—Left subclavian artery and left auricle; mitral leak established at upward arrow, and abolished at downward arrow.

evident when the lesion occurs; but tricuspid incompetence is not aggravated in the same way by anoxemia as is mitral incompetence. The right ventricle, too, seems to me to resist mechanical interference better than the left, which has gone into fibrillation on many occasions for apparently trivial causes. Because of this tendency of the left ventricle, ventricular manometers, previously used for determining intraventricular changes of pressure, have been abandoned for these experiments and arterial manometers adopted. Even though the ventricles withstand the manometers and seem to act vigorously after their insertion, the functioning power of the heart is not the same as when they are avoided. The pericardium also has to be interfered with for the insertion of ventricular manometers and, as already stated, it is better to preserve it.

Notwithstanding the minor influence of tricuspid leak, it is important to ascertain whether tricuspid insufficiency is, as some observers hold, a frequent sequel to mitral defect or not. The question reappears in section 3, in connection with that of how the venous pulse is affected by left side lesions. Here it is sufficient to state that never in these experiments has a picture presented by the right auricle as a result of mitral defect at all resembled that presented by direct tricuspid leak experimentally established, as seen in the records (fig. 5). Of course, failure to produce this secondary effect of mitral leak lasting from fifteen to twenty minutes at the outside does not connote failure to produce it as a result of mitral defect lasting from fifteen to twenty weeks or months. Yet it is a striking fact that with marked hindrance to outlet on the left side, accumulation in the left side of the heart and pulmonary engorgement, the tricuspid valve remains competent. When the pressure rises in the right ventricle, the systole of this chamber does coincide with a rise in the corresponding phase of the intra-auricular curve, and there may be a small reflux through the valve, but it is not detectable by ordinary means, such as the manometer and palpation for thrill.

SECTION 3

Although the venous pulse record has in recent years become a sort of sheet anchor in the diagnosis of cardiac lesions and the determination of functional capacity of the heart mechanism, it still presents many features, certain oscillations and pressure changes, especially in optical records, the significance of which is by no means understood. A knowledge of these, however, and of the way in which they are affected by cardiac defects is most important for the interpretation of irregular action. It will be best to consider briefly at this stage the various elements constituting the venogram, the three classic waves, *a*, *c* and *v*, with the corresponding dips or depressions. The *a* elevation is generally looked on as being associated with auricular contraction; but the

mechanism of its production, whether by reflux or by rebound from a closed or partially closed venous orifice is not agreed on. Accepting the influence of reflux in its formation—and no one can well doubt it who has observed the filling with auricular systole of the cardiac end of one of the large veins when clamped a few centimeters from the orifice—it is still difficult to say how far the size of *a* is to be taken as a measure of the force of auricular contraction. In general terms, it may be asserted that with increased pressure in the right auricle, whether caused by augmented inflow or by pulmonary or mitral obstruction, the size of *a* is increased and its measurement gives some indication of auricular force and intra-auricular pressure. The height of the *a* summit varies with respiration, being highest after the end of inspiration when intra-thoracic pressure is low and venous inflow is free. This does not apply to conditions with the chest open. Individual peculiarities which give rise to different types of venous pulse are evident only with the closed thorax.

Variations in the size of the *a* wave and in its position relative to the other elements may occur independently of respiration and of intra-thoracic pressure changes. The question may be supposed to lose in value in light of the view held by some observers, that the auricle is of little consequence as a driving or dynamic factor in the circulatory mechanism, a view which seems to be based on the weakness of the walls of the chamber and on the resistance opposed to the auricular output by the filled or nearly filled ventricle, as well as the facility with which reflux may occur into large veins of low resistance. Feil and Katz [9] have demonstrated, however, that when the auricles have been made to fibrillate artificially the output is always less than in periods of normal auricular activity. The results certainly point to the possession of dynamic influences by the auricles. It will be shown, presently, that the *a* wave, or its equivalent in intra-auricular curves, undergoes some increase in size as a result of increased pressure in either auricle.

That part of the auricular record which represents the *c* wave of the venous record usually consists of two or three vibrations, the first of which corresponds to the *c* wave proper and precedes the arterial upstroke by one-twentieth of a second. It is of little significance in experimental work beyond showing that an early or presphygmic reflux may occur as indicated by a rise of pressure beginning at this point. The dip succeeding the *c* elevation in the venous pulse, referred to as the *x'* depression, generally indicates the lowest point of venous pressure when the chest is closed, and from this point the static rise of venous pressure begins. The position of this dip *x'* relative to the *T* deflection of the electrocardiogram seems to me to have some significance as revealing cardiac functional capacity, and it has been dealt with in a

9. Feil, H., and Katz: Proc. Soc. Exper. Biol. & Med. **20**:323, 1923.

previous publication.[10] A well marked rise of the point may be observed in the records of tricuspid regurgitation, and this appearance is worthy of attention when such a lesion is suspected. The venous pulse has sometimes been taken from the inferior vena cava in these experiments by the insertion of manometers. The record then resembles that taken from the auricular appendix, so that the latter gives a picture which may be interpreted in terms of venous pulsation in general.

Lastly, the form of the *v* wave may give important information regarding efficiency and type of lesion in a given condition. A special oscillation already mentioned occurs near the summit of this wave, which has been described in optical venograms from a man as coinciding with the beginning of diastole before the opening of the auricula-

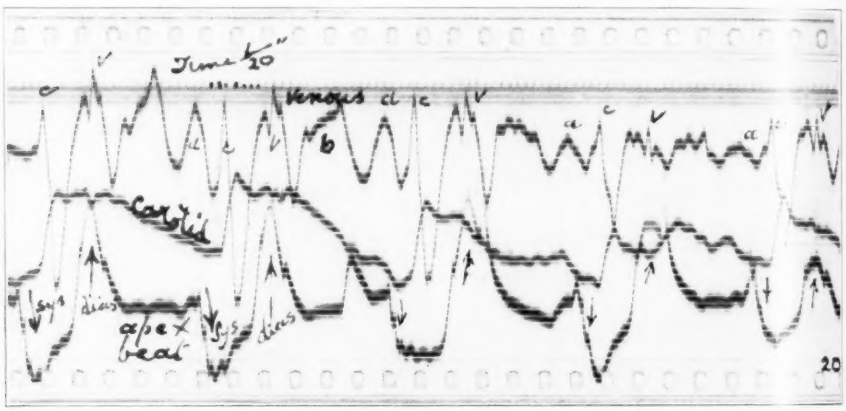

Fig. 7.—Triple record from normal man; venogram (top); carotid (middle); apex beat (below); this record shows the well marked diastolic stroke against the chest tambour, with sudden rise of intrathoracic pressure after systole; the downward arrow is at the beginning of systole, the upward arrow in diastole; further description is given in the text.

ventricular valves. An alternative or additional cause of this oscillation to that already mentioned (closure of the semilunar valves) is suggested by records such as that presented in figure 7. This is a triple record: venogram (top), carotid (middle) and apex beat (bottom). The apex beat is an inverted cardiogram taken in reality to the right of the apex beat in a young man. There is a downward deflection of the beam of light from the mirror on the recording tambour at the onset of systole, that is, an expression of the negative pressure in the thorax, accompanying systole (due to the tug of the right ventricle in

10. Barry, D. T., and O'Donovan, W. J.: Arch. int. de med. exper. **2**:311, 1926.

rotation of the heart). There is a sharp upward deflection at the onset of diastole, that is, an expression of the sudden increase of intrathoracic pressure which marks this phase. The apex of this sudden upward deflection corresponds with the sharp oscillation of the *v* wave in the venogram above it, both points corresponding closely with the dicrotic notch of the arterial curve; but it is almost certain that the closure of the semilunar valve which coincides with it could not occasion a deflection of this sort in the curve of the apex beat. Systole is marked in the record by a downwardly directed arrow, diastole by an upwardly directed one. Consequently there is a sudden sharp increase of intrathoracic pressure at the inception of diastole which can only be yielded by a sudden sharp increase in the volume of the heart. Diastole occurs rapidly, if not actively, and is not the gradual process which it is generally considered to be.

Patterson and Starling stated that not only is there no sharp recoil of ventricular muscle with beginning diastole, but also that if blood is not present to distend the fibers, and contractions continue, the volume of the heart will get smaller and smaller. But an empty heart suddenly cut out of the thorax of a cat or rabbit, keeps on beating, contracting and expanding, for some time. Such curves as that in figure 7 can only be explained by a fairly sharp expansion of the heart chambers. It may be that cardiac muscle is slow to return to the relaxed condition after contraction, but it must be remembered that there is a ring, or rings, of rigid fibrous tissue in the walls which certainly gives a sharp recoil after compression, a recoil which must cause a sudden change in pressure within the closed space surrounding it.

During the influence of the mitral lesion the pressure in the left auricle is high in both systole and diastole, and remains higher than before during at least a few cycles after the lesion. The high mean pressure in the left auricle produces some increase of pressure in the right auricle, apparently by direct influence, before there is any effect from pulmonary stasis. In the second beat after the mitral lesion the right auricle record shows a change, a rise in level (fig. 2), which is intensified and outlasts the lesion when arrhythmia is a sequel (figs. 3 and 4). This aspect of the problem has to be developed further before any definite statements are made concerning the particular phases of the venous pulse affected by mitral insufficiency; but the general statement holds, namely, that the venous pulse as indicating right auricle pressure changes does vary in its characters with left auricle pressure changes caused by mitral valvular defect.

RESULTS

The methods employed in this work are, of course, open to criticism from different points of view. Firstly, there is the condition

of the open thorax, which has been already dealt with to some extent. The normal negative pressure to which the heart is subject in the closed thorax cannot be reproduced in artificial conditions. Efforts to do so have been made in a different form of work,[5] but when a tube must be introduced into the organ it is impossible. The presence of such a tube is naturally objectionable for investigation, but a better method of producing valvular defect does not suggest itself. From the comparisons of output, before and after introduction, it is obvious that the tube causes even less interference with function than does a manometer in the ventricle. Generally speaking better conditions are not available for the investigation than those chosen, and every effort has been made to imitate natural conditions.

Although some discrepancies for the measurement of output in the results of mitral lesion were observed, there was, on the whole, a remarkable uniformity in the estimations for similar conditions, which inspires a certain amount of confidence in applying the results to natural conditions. The function of the heart, when hampered by a defective mitral valve, has been shown to be decidedly diminished in most circumstances. This is aggravated by an increase in the amount of venous inflow. Function decreased to a much greater extent in the case of a mitral lesion than in the case of a tricuspid lesion, in otherwise similar circumstances. Increase of arterial resistance does not add to the deficiency resulting from tricuspid defect proportionately to the depressing influence of rising resistance in the case of mitral defect. An increase of venous inflow does cause an immediate improvement in the function under the influence of tricuspid defect. The effect produced in the pulmonary circulation by failure of the mitral valve adds considerably to the gravity of the condition, because with this there is reduction of aeration of the blood, and this has been shown to cause a marked deterioration of muscular efficiency. The influence of this factor is not so obvious in right side lesions.

Compensation of a valvular defect is supposed to be brought about by the mechanism which enables the heart to yield increased work in physiologic conditions. This view has been put forward by Starling and others.[11] When the ventricle dilates, owing to a greater volume of blood within it, the stretching of the fibers causes enhanced contractions. But it is necessary to bear in mind the state of the muscle, the energy behind the stroke, so to speak. Hypertrophy provides for this increased demand, hypertrophy with good metabolism. There are limitations to this so-called law of the heart in directing compensation. We have seen what happens under strain when the pericardium is removed; not only, then, is undue distention not responded to by

11. Starling, E. H.: Presse méd. **60**:29 (July) 1922.

enhanced contraction, but also the stretching is a cause of inefficient contraction, and this occurs at a stage before the limits of elasticity or extensibility are passed, as is shown by recovery of contractile power and efficiency in many organs when favorable conditions are restored, after tests in which there was practically no function and the distention of the chambers was great. It was surprising to note the effect of leak into the auricle when a heart without pericardium was acting with large flow against high resistance. The lesion proved to be the last straw which turned the scale, and not only during, but after, lesion the heart refused to function anything like equivalent to the prelesion standard, not at least until resistance to output was reduced.

In disease in man the onset of defects is slow, and in many ways not comparable to the conditions caused in experiment; but in man exacerbations occur, sudden strain, etc., to which the lessons taught by experiment may be applied; and these lessons indicate, above all attention to the peripheral resistance and to the aeration of the blood.

The view put forward by Whitney [12] that the factor of most importance in anoxemia is that of the circulation, those of the respiratory exchange and the blood composition being of secondary consequence, has been opposed by Schneider and Truesdell.[13] The objection is that the respiratory and blood influence is permanent. It is clear from the present experiments that the force and work of the heart is quickly affected in anoxemia. Valvular incompetence is certainly a suitable condition for a demonstration of this effect. Anoxemia produces its effect on the musculature, its influence being apparent, especially with valvular lesions, and the figures given for the changes wrought leave little doubt of the disturbance.

The influence of mitral regurgitation on right intra-auricular pressure curves, though not pronounced, is distinct. It is always revealed by the second regurgitant beat as a rise in general level. The auricular systolic wave rises appreciably higher than in the nonlesion beats, and the succeeding oscillations, synchronous with early ventricular systole, are also raised to a higher level (figs. 2, 3 and 4). Obviously, the right auricle was somewhat encumbered by the abnormal phase, and it was suddenly relieved when this ceased.

CONCLUSIONS

The work of the heart when hampered by a defective mitral valve is distinctly diminished in most circumstances. This deficit is made more evident when the venous inflow is increased, and it is decidedly aggravated by increases of peripheral resistance.

12. Whitney, J. L.: Aviation, J. A. M. A. **71**:1390 (Oct. 26) 1918.
13. Schneider, E. C., and Truesdell, D.: Am. J. Physiol. **71**:90, 1924.

The results of changing the artificial resistance suggest the importance of noting the degree of rigidity and obstruction in arterioles as compared with mean arterial pressure in man, and ascertaining the pressure gradient.

The defect in aeration of the blood occurring with mitral lesion has a deleterious action on the cardiac muscle; and when anoxemia is set up by defective pulmonary ventilation the deficiency in compensation of a mitral lesion is marked.

In lesions of the tricuspid valve the influence of a rise in arterial resistance is slight; that of anoxemia is not at all comparable with its effect in mitral incompetence.

The fall in arterial pressure with tricuspid lesions is small and of slow onset; that with mitral lesions is marked and of rapid onset.

Tricuspid deficiency does not occur in experiment as a result of a mitral lesion.

Tricuspid incompetence causes well-marked changes in the records of the right auricle, with a rise of pressure and characteristic vibrations.

Mitral incompetence causes changes in the records of the right auricle which are less characteristic than those on right side lesion. Further investigation of these changes is necessary, and there is not any immediate prospect of my being in a position to make it.

DISEASES OF THE LIVER

VII. FURTHER STUDIES IN EXPERIMENTAL OBSTRUCTIVE JAUNDICE *

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In a previous paper we have reported the effect of experimental obstructive jaundice on hepatic function as measured by certain tests.[1] At that time particular attention was paid to the changes in the fructose tolerance, the nitrogen partition in the blood, the serum bilirubin and the phenoltetrachlorphthalein test.[2] We now wish to report further studies with particular reference to the bromsulphalein test of Rosenthal and White,[3] the bile acid content of the blood and the elimination of intravenously injected bile acids. The serum bilirubin was followed as an index to the degree of jaundice, a standard of reference being thus furnished for the comparison of the results in the two series of experiments.

Two groups comprising a total of fifteen animals were studied: those in which the common duct had been ligated, and those in which cholecystectomy had been performed in addition to ligation of the common duct. In all cases complete studies with regard to the tests to be employed were made before the operation and at intervals of from one to three days during the period following obstruction. Cholecystenterostomy to relieve the obstruction was successfully performed in two instances and the course of recovery studied.

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* From the Division of Medicine, Mayo Clinic and The Mayo Foundation.

* The data presented in this paper are taken from a thesis submitted by A. M. Snell to the Faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of M. S. in Medicine; reported in part before the annual meeting of the American Society for Clinical Investigation, Atlantic City, 1926.

1. Snell, A. M.; Greene, C. H., and Rowntree, L. G.: Diseases of the Liver: II. A Comparative Study of Certain Tests for Hepatic Function in Experimental Obstructive Jaundice, Arch. Int. Med. **36**:273 (Aug.) 1925. Greene, C. H.: Aldrich, Martha, and Snell, A. M.: Studies in Obstructive Jaundice, J. Clin. Investigation. **2**:604, 1926.

2. Greene, C. H.; Snell, A. M., and Walters, Waltman: Diseases of the Liver: I. A. Survey of Tests for Hepatic Function, Arch. Int. Med. **36**:248 (Aug.) 1925.

3. Rosenthal, S. M.: Clinical Application of the Bromsulphalein Test for Hepatic Function, J. A. M. A. **84**:1112 (April 11) 1925. Rosenthal, S. M., and White, E. C.: Studies in Hepatic Function: VI. A. The Pharmacological Behavior of Certain Phthalein Dyes. B. The Value of Selected Phthalein Compounds in the Estimation of Hepatic Function, J. Pharmacol. & Exper. Therap. **24**:265, 1924.

The animals were kept on a constant diet of meat, milk and dog biscuit. Following the production of obstruction, the changes in the general condition of the animals were essentially identical with those reported in the preceding paper. Anorexia, weakness and progressive loss of weight were constant. To combat this, certain animals were fed daily by stomach tube with a mixture of corn syrup and milk. This served to prevent the immediate postoperative loss in weight, and the dogs remained in better condition than when allowed to eat at will. These animals remained alive and in good condition for a much longer time than those in the first series of experiments. Two survived for periods of 108 and 120 days, respectively, and in these it was possible to study the late effects of complete biliary obstruction.

ILLUSTRATIVE PROTOCOLS

The functional disturbances observed following the production of obstruction to the bile passages were strikingly uniform in both series of experiments. Complete studies were made on animals with obstructive jaundice both in the presence and in the absence of the gallbladder. The four protocols which follow are characteristic of the fifteen animals studied. The changes in an additional experiment are given in tabular form only.

Experiment 1.—The dog was an adult female, black and brindle mongrel in good condition, weighing 10 Kg. On Oct. 21, 1925, a complete series of functional tests was made; on October 29, under ether anesthesia and with aseptic technic cholecystectomy was performed and the common duct doubly ligated and severed between clamps. The following day the sclerotics and mucous membranes were subicteric, and there was bile in the urine; the functional studies were repeated. On November 1, jaundice was marked, and the stools were light colored. The animal had recovered from the immediate effects of operation and was in good condition. The functional studies are shown in table 1 and in figure 1. This animal was tube-fed on corn syrup and milk, and remained in fairly good condition. The icterus gradually decreased in intensity, and the animal lost weight slowly. After about eight weeks, evidences of collateral circulation appeared on the abdominal wall, and there was also definite distention of the abdomen. On January 1, ascites could be demonstrated; this increased rapidly, and in consequence the weight of the animal showed a marked rise. Figure 2 shows the extent of the collateral circulation in this animal. On February 1, the weight was 11.6 Kg.; there was marked edema of the abdominal wall and hind legs. This edema increased progressively up to the time of death, which occurred on Feb. 14, one hundred and eight days after the operation.

At necropsy there was marked edema of the abdominal wall and hind legs; 3,800 cc. of ascitic fluid was removed from the abdominal cavity. Collateral circulation was well developed, particularly in the vessels of the omentum, which was adherent to the laparotomy scar. The veins of the lower part of the esophagus, the gastric collateral circulation, the veins of Sappey, the splenic vessels and those of the pelvic plexus were dilated. There was only slight staining of the tissues with bile, and the ascitic fluid contained only small quantities of bilirubin. The liver was small, shrunken, tough and reddish-brown with a finely granular mottled surface. There were adhesions of the duodenum to the fossa of the gallbladder. The common duct was greatly distended, being about 2 cm. in diameter, and contained a grayish, mucoid fluid. Obstruction to the duct was complete.

TABLE 1.—*Changes Observed Following Ligation of the Common Bile Duct and Cholecystectomy (Experiment 1)*

	Time After Obstruction, Days	Weight, Kg.	Hemoglobin, Gm.	Nonprotein Nitrogen, Mg., Per Cent	Blood Urea, Mg., Per Cent	Serum Bilirubin, Mg., Per Cent	Van den Bergh Direct Reaction	Bile Acids, Mg., Per Cent	Bromsulphalein Test: Dye in Serum, Per Cent: 5 Minutes	10 minutes	15 Minutes	1 Hour	2 Hours
1925													
10-21	6	10.2	....	25	22	0.0	..	6.5	20	18	4	1	0
10-29				Ligation of common bile duct and cholecystectomy									
10-30	1	10.0	....	40	23	3.0	+	9.7	56	40	32	24	14
10-31	2	10.0	....	22	22	4.0	+	9.1	40	32	30	18	16
11-2	4	10.0	....	24	13	7.7	+	13.6	32	28	24	16	8
11-4	6	....	....	40	26	8.7	+	24.0	52	48	44	26	14
11-7	11	....	....	31	25	7.3	+	17.5	64	48	40	24	16
11-11	15	9.6	....	22	12	5.5	+	11.5	64	48	48	32	24
11-16	20	9.4	....	30	15	4.4	+	11.3	60	48	40	32	12
11-17	26	....	12.5	24	11	4.4	+	13.4	60	52	48	28	20
12-	33	9.3	12.7	27	14	4.7	+	17.2	64	56	56	26	16
12-	40	....	10.9	32	17	4.2	+	13.3	64	56	52	32	24
12-	47	8.5	11.5	22	7	3.6	+	11.7	60	60	56	36	24
12-	53	7.9	9.8	28	20	4.0	+	7.4	44	40	32	24	24
12-	60	7.8	9.6	30	15	3.4	+	8.3	80	56	40	30	16
1926													
1-	67	10.3	8.8	23	..	2.8	+	5.8	44	44	40	30	20
1-	75	10.4	8.3	30	30	2.2	+	8.9	80	60	56	22	32
1-	82	10.4	8.9	22	12	2.5	+	9.3	76	40	32	28	24
1-	91	10.6	8.1	..	16	2.0	+	5.5	60	40	30	22	40
2-	95	11.6	6.7	27	21	2.1	+	5.1	40	30	30	24	14
2-	102	12.2	6.4	21	..	2.2	+	6.1	76	44	38	30	20

Ascites present.

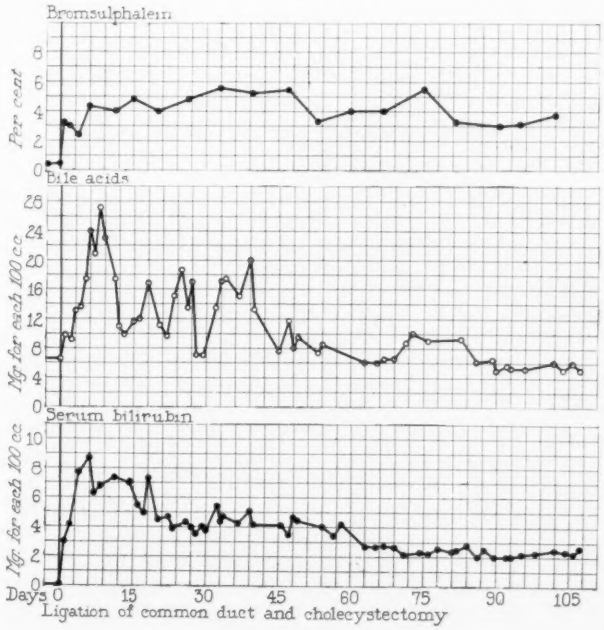
Bromsulphalein
Per cent
Bile acids
Mg. for each 100 cc.
Serum bilirubin
Mg. for each 100 cc.
Days 0 15 30 45 60 75 90 105
Ligation of common duct and cholecystectomy

Fig 1 (experiment 1).—Changes in bromsulphalein test, bile acid reading and serum bilirubin following ligation of the common bile duct and removal of the gallbladder.

Microscopic examination of the liver showed the presence of chronic cholangeitis with thickening of the walls of the bile ducts and infiltration with small lymphocytes. There was fibrosis of the terminal bile ducts and evidence of some regeneration of the ducts. Moderate diffuse infiltration of the parenchyma with lymphocytes was noted. The general appearance was that of monolobular biliary cirrhosis.

Experiment 2.—The dog was an adult female mongrel in good condition, weighing 13.5 Kg. On March 24, 1926, a control series of functional tests was performed. On March 26, under ether anesthesia and with aseptic technic, the common duct was doubly ligated and severed between clamps. The gallbladder was removed at the same time. An increase in the bile salts in the blood was noted one hour later and in the serum bilirubin within four hours. The following

TABLE 2.—*Changes Observed Following Ligation of the Common Bile Duct and Cholecystectomy (Experiment 2)*

Date	Time After Obstruction, Days	Weight, Kg.	Hemoglobin, Gm.	Nonprotein Nitrogen, Mg., Per Cent	Blood Urea, Mg., Per Cent	Serum Bilirubin, Mg., Per Cent	Van den Bergh Direct Reaction	Bile Acids, Mg., Per Cent	Bromsulphalein Test: Dye in Serum, Per Cent: 5 Minutes	10 Minutes	15 Minutes	1 Hour	2 Hours
1926													
3-24....	0	13.4	16.4	39	48	0.0	0	6.8	20	8	4	0	0
3-25....	0			Ligation of common duct and cholecystectomy									
3-26....	$\frac{1}{3}$	13.1	....	..	..	1.6	+	23.2	36	30	30	22	21
3-27....	1	12.6	16.9	30	21	6.1	+	17.4	72	64	56	40	4
3-29....	3	....	15.4	32	17	11.0	+	17.6	80	72	64	40	8
3-31....	5	....	13.5	32	17	23.1	+	33.0	80	72	60	44	4
4-2.....	7	....	....	..	..	22.3	+	22.6	90	84	78	52	4
4-5.....	10	....	12.0	33	13	16.9	+	11.8	72	66	60	40	4
4-13....	18	12.0	15.3	44	50	6.5	+	14.3	56	56	40	32	4
4-19....	24	11.9	....	..	..	4.8	+	11.3	64	56	56	44	..
4-27*...	33	....	14.3	27	15	2.3	+	6.9	50	40	30	..	8
5-5.....	40	....	....	..	..	1.6	+	6.9	64	64	56	20	8
5-11....	41	....	13.0	28	20	1.6	+	13.7	66	50	..	16	8
5-18....	53	13.3	16.4	33	17	2.5	+	15.8	60	50	32	16	8
6-3†....	69	9.4	14.8	22	12	2.4	+	8.3	40	32	32	18	8
6-8.....	74	9.2	12.8	24	28	3.8	+	9.6	..	44	40	20	16
6-14....	80	10.0	12.3	27	19	2.5	+	12.6	66	66	64	28	8
6-21....	87	9.6	....	27	23	2.0	+	11.4	56	40	40	24	6
6-28....	94	8.8	11.4	22	13	1.4	+	13.0	64	40	40	28	8
7-6.....	102	9.6	11.6	23	11	1.5	+	7.6	80	56	56	32	8
7-9.....	105	10.3	11.2	24	15	1.4	+	7.8	64	40	40	20	6
7-19....	115	12.7	10.0	21	30	1.6	+	10.0	60	60	40	32	0
7-26‡...	122	12.6	5.1	23	10	2.0	+	7.9	56	54	50	40	14
7-31....	127	11.1	3.7	..	34	....	+	4.0	60	60	54	32	..

* Ascites present. † Paracentesis, 4,000 cc. ‡ Paracentesis, 2,000 cc.

day the clinical signs of icterus were evident, and the urine contained bile. The animal recovered rapidly from the effect of the operation and remained in good condition, although it lost weight slowly. The changes in the laboratory tests are shown in table 2 and in figure 3. The icterus gradually decreased in intensity, although the urine contained bile pigment and the stools remained light colored. Abdominal distention was noted, and on April 27, thirty-three days after the production of obstruction, free fluid was demonstrated in the abdomen. The ascites gradually increased, and on June 3 it was so great as to cause respiratory embarrassment. Paracentesis was performed, and 4 liters (equivalent to nearly a third of the weight of the animal) of bile-stained fluid was removed. The animal remained in fairly good condition for a fortnight, when the abdomen again began to enlarge. At this time the superficial abdominal veins were dilated, and there was a widespread collateral circulation. The appetite was

poor, and, although the body weight increased, the dog rapidly lost flesh and strength. On July 26, 2 liters of clear, straw-colored ascitic fluid was removed by paracentesis. Relief was temporary, and the dog died five days later, one hundred and twenty-seven days after the obstruction to the common bile duct.

At necropsy the liver was greatly shrunken, firm and bile-stained. The surface was covered with old organized adhesions, many of which were vascularized. The obstruction to the common duct was complete. It was greatly dilated and filled with a grayish mucoid secretion. The intestines were free from bile pigment. The micropscopic appearance of the liver was essentially the same as in the preceding experiment.

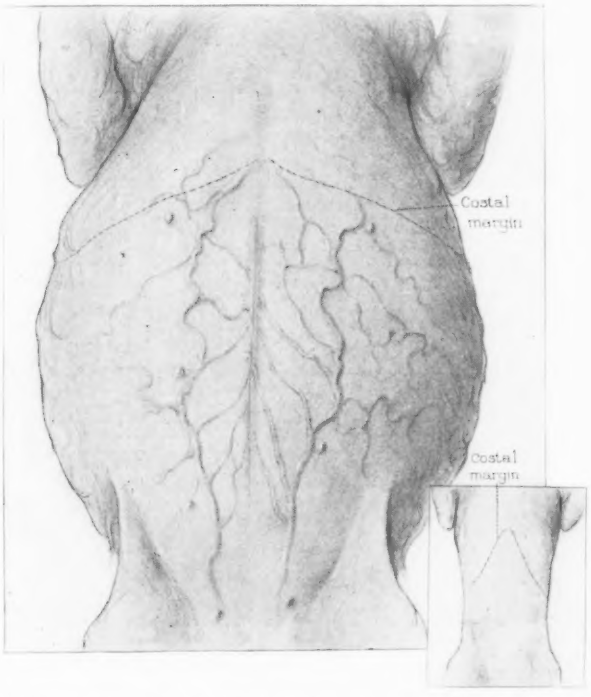

Fig. 2 (experiment 1).—Abdominal ascites and extensive collateral circulation in a dog nine weeks after ligation of the common bile duct.

Experiment 3.—The dog was an adult male, brown and white terrier in good condition, weighing 11.4 Kg. On October 21, 1925, a complete series of functional tests was performed. On October 29, under ether anesthesia and with aseptic technic, the common duct was doubly ligated and severed. The gallbladder was not disturbed. The following day the animal was in good condition without marked postoperative reaction. The functional studies were repeated. On November 1, slight icterus was apparent in the mucous membranes and conjunctiva; the stools were clay colored. The animal was given corn syrup and milk daily by stomach tube, and thereafter remained in good condition with only slight loss of weight. The results of the functional tests are shown in table 3 and in figure 4.

On December 18 an attempt was made to relieve the obstruction. The animal was slightly jaundiced at this time, but careful examination at operation proved that the obstruction was complete. A quantity of thick, black, viscid bile was removed from the gallbladder by means of a trocar, and cholecystoduodenostomy was performed. For the first few days after the operation, the stools were alternately light and dark, but subsequently they uniformly contained bile. The animal remained in good condition thereafter. On January 1, the stools again showed evidence of intermittent obstruction, although the serum bilirubin varied only slightly. The obstruction later became complete, and bile was not apparent in the stools.

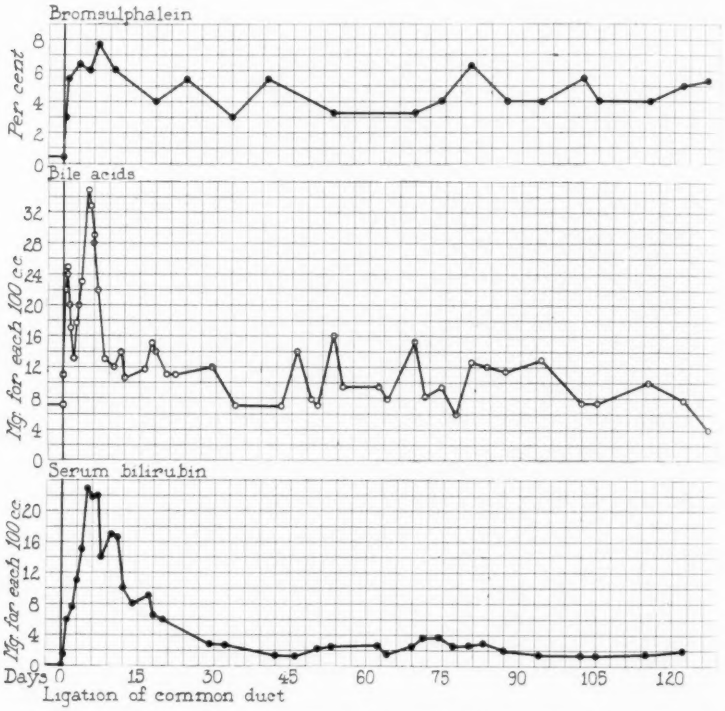

Fig. 3 (experiment 2).—Changes in bromsulphalein test, bile acid reading and serum bilirubin following ligation of the common bile duct and removal of the gallbladder.

On March 18, the animal was killed, and complete obstruction from postoperative stricture at the site of the anastomosis was found. The liver was small, firm and granular, with definite evidence of biliary cirrhosis.

Experiment 4.—The dog was an adult female, mongrel collie, in good condition, weighing 11.5 Kg. On Jan. 6, 1926, a control series of functional tests was performed. On January 14, under ether anesthesia and with aseptic technic, the common duct was doubly ligated and severed between clamps. The gallbladder was not disturbed. Two days later, the animal was in good condition without marked postoperative reaction; the functional studies were

repeated. The serum bilirubin was 0.7 mg., and the next day icterus was apparent in the skin and mucous membranes. The animal was fed dog biscuit and meat with the addition of corn syrup and milk, and remained in good condition without significant loss of weight. The results of functional tests are given in table 4 and in figure 5.

On Feb. 16, 1926, under ether anesthesia and with aseptic technic, an attempt was made to relieve the obstruction. A quantity of thick, black, viscid bile was removed from the gallbladder by means of a trocar, and cholecystoduodenostomy was performed. Bile appeared in the stools the following day. Drainage was satisfactory for a short time, but within three weeks the color of the stools

TABLE 3.—*Changes Observed Following Ligation of the Common Bile Duct (Experiment 3)*

Date	Time After Obstruction, Days	Weight, Kg.	Hemoglobin, Gm.	Nonprotein Nitrogen, Mg., Per Cent	Blood Urea, Mg., Per Cent	Serum Bilirubin, Mg., Per Cent	Van den Bergh Direct Reaction	Bile Acids, Mg., Per Cent	Bromsulphalein Test: Dye in Serum, Per Cent: 5 Minutes	10 Minutes	15 Minutes	1 Hour	2 Hours
1925									10	4	4	1	0
21....	0	11.4	....	17	23	0.0	0	6.5	16	8	4	0	0
29				Ligation of common bile duct									
30....	1	11.4	....	26	20	0.0	0	6.9	16	8	4	2	0
31....	2	....	....	36	12	0.0	..	6.7	20	10	10	8	6
2.....	4	11.3	....	27	17	3.3	+	12.2	22	20	18	16	12
4.....	6	....	....	27	15	5.9	+	16.8	56	48	40	24	12
9.....	11	....	....	40	23	5.5	+	23.1	72	56	40	20	16
13....	15	11.1	....	26	9	4.6	+	17.2	80	80	56	24	18
18....	20	10.8	....	31	15	3.7	+	18.2	56	44	40	20	18
24....	26	....	11.4	35	13	4.2	+	12.7	60	48	44	28	16
1.....	33	10.6	11.1	42	11	4.3	+	21.4	64	52	40	24	14
5.....	37	....	11.4	31	18	4.6	+	30.8	64	56	48	28	20
8.....	40			Cholecystoduodenostomy									
9.....	1	10.5	10.8	30	25	5.3	+	14.2	44	40	32	18	14
10....	2	....	10.3	25	10	4.4	+	....	54	48	44	24	16
11....	3	10.7	8.7	25	13	...	..	....	64	52	48	28	16
12....	4	....	8.6	28	13	3.6	+	13.6	64	44	40	20	16
15....	7	10.8	8.3	20	9	3.8	+	15.0	80	64	48	32	24
18....	10	10.8	....	12	10	3.5	+	16.0	68	64	40	32	18
21....	13	10.5	8.2	30	18	3.6	+	19.4	56	48	48	32	24
28....	20	10.0	7.1	36	21	3.2	+	....	72	56	40	30	16
1926													
4.....	27	10.9	8.8	37	20	2.8	+	25.0	80	48	40	24	20
12....	35	11.0	9.5	33	22	2.1	+	26.7	60	40	36	20	16
19....	42	11.2	10.1	21	11	2.6	+	30.6	60	38	30	22	14
26....	49	11.0	9.3	22	11	3.3	+	8.7	58	38	30	22	16
1.....	55	10.8	10.4	31	19	2.9	+	11.7	60	36	32	28	20
8.....	62	10.7	10.6	30	20	3.8	+	17.5	76	30	30	32	20
15....	69	10.6	11.5	..	15	3.7	+	15.5	54	38	30	28	20
23....	77	10.7	9.7	26	21	2.9	+	13.1	38	30	22	16	14
1.....	83	10.8	11.4	11	..	4.2	+	15.8	48	44	36	24	16
9.....	91	....	12.5	11	11	3.8	+	23.8	38	36	32	24	20
18....	100	....	9.3	10	13	2.5	+	21.4	38	28	24	24	24

gave evidence of intermittent obstruction, which later became complete. The animal was killed on May 4, seventy-six days after the second operation. The liver was shrunken and contracted, and the gallbladder and bile ducts were dilated and contained a grayish, mucoid fluid. The opening between the gallbladder and the duodenum had closed, and obstruction was complete.

DISCUSSION OF TESTS

Bile Pigments.—In our previous paper dealing with experimental obstructive jaundice,[1] particular emphasis was placed on the changes in the serum bilirubin during the first two weeks after the production

of biliary obstruction. Particularly interesting were the differences noted in the two groups of animals with reference to the influence of the gallbladder in delaying the development of bilirubinemia; this observation was recently stressed by Mann and Bollman.[4] Similar and confirmatory results were obtained in this series of experiments.

The maximal degree of bilirubinemia occurred between the first and second week in both series of animals. Thereafter the serum bilirubin gradually decreased, even though complete obstruction was maintained. This is well shown in experiments 1 and 2, in which the

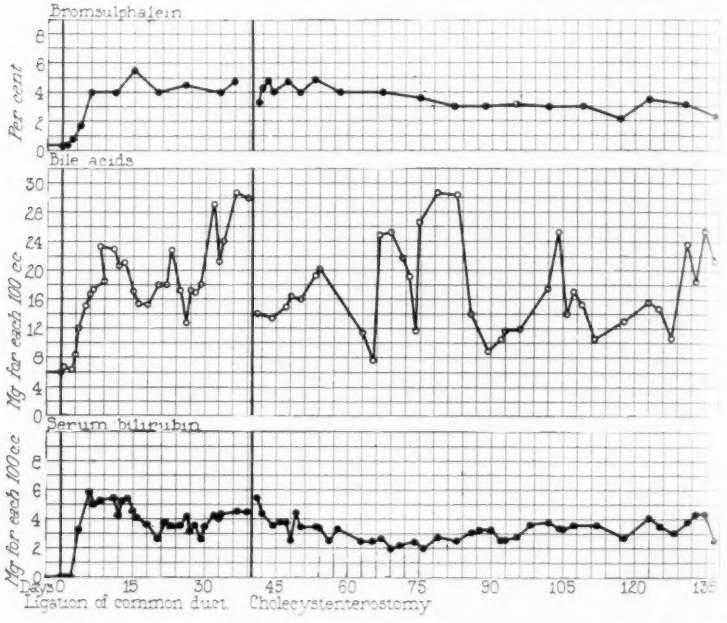

Fig. 4 (experiment 3).—Changes in bromsulphalein test, bile acid reading and serum bilirubin following ligation of the common bile duct. Cholecystoduodenostomy was performed on the fortieth day after obstruction.

serum bilirubin decreased spontaneously from 8.7 to 2 mg., and from 23.1 to 1.4 mg., respectively. Associated with the decrease in the serum bilirubin there was a corresponding decrease in the clinical evidence of icterus.

The effect of cholecystenterostomy and consequent reestablishment of biliary drainage apparently varies with the duration of the obstruc-

4. Mann, F. C., and Bollman, J. L.: The Relation of the Gallbladder to the Development of Jaundice Following Obstruction of the Common Bile Duct, J. Lab. & Clin. Med. **10**:540, 1925.

tion. With obstruction lasting thirty days or longer, as in these experiments, there was no immediate change in the degree of bilirubinemia after the relief of the obstruction. Spontaneous closing of the fistula made it impossible to study the effect of long-continued drainage in such cases.

Bromsulphalein.—Bromsulphalein [5] (the disodium salt of phenoltetrabromphthalein disulphonic acid) was used in the present series of experiments in place of the phenoltetrachlorphthalein previously studied.

TABLE 4.—*Changes Observed Following Ligation of the Common Duct and Cholecystectomy (Experiment 4)*

Date	Time After Obstruction, Days	Weight, Kg.	Hemoglobin, Gm.	Nonprotein Nitrogen, Mg., Per Cent	Blood Urea, Mg., Per Cent	Serum Bilirubin, Mg., Per Cent	Van den Bergh Direct Reaction	Bile Acids, Mg., Per Cent	Bromsulphalein Test: Dye in Serum, Per Cent — 5 Minutes	10 Minutes	15 Minutes	1 Hour	2 Hours
1925													
6.....	0	11.5	16.2	28	23	0.0	0	3.8	14	6	2	0	0
14.....	0				Ligation of common bile duct								
16.....	2	10.7	16.4	18	34	0.7	+	13.1	32	32	30	24	20
18.....	4	10.5	15.0	21	16	2.8	+	15.5	64	56	32	20	10
21.....	7	10.3	....	..	..	4.9	+	10.3	64	60	40	32	20
23.....	9	10.3	....	..	..	3.8	+	7.2	76	72	48	32	20
28.....	14	10.4	14.3	12	16	4.6	+	6.7	76	72	32	28	24
1.....	18	10.4	13.0	35	26	4.5	+	7.7	60	40	30	32	14
8.....	25	10.3	12.0	21	18	4.6	+	12.9	76	60	40	30	20
15.....	32	10.2	....	13	11	2.8	+	6.9	76	60	30	32	14
16.....	33				Cholecystoduodenostomy								
20.....	4	9.2	12.6	..	..	3.2	+	....	40	30	22	16	10
22.....	6	9.1	....	..	..	3.0	+	....	40	30	22	16	10
24.....	8	....	12.0	22	19	2.2	+	9.3	40	30	28	16	10
1.....	13	9.4	11.7	..	..	1.9	+	7.4	30	22	16	10	10
8.....	20*	9.2	12.0	20	13	1.9	+	9.2	40	28	20	16	16
16.....	28	9.2	12.0	21	14	0.8	+	5.7	30	22	16	12	10
22.....	..	9.2	....	..	..	0.4	+	6.6	40	26	12	10	4
30.....	41	9.3	12.7	39	17	0.3	+	8.2	36	32	32	16	12
5.....	47	9.7	13.0	35	18	0.3	+	3.6	36	20	14	10	6
13.....	55	9.4	14.8	35	..	0.9	+	5.3	50	50	32	28	20
19.....	61	9.7	....	27	20	1.7	+	8.3	60	48	40	30	20
27.....	69	....	....	32	29	2.8	+	5.6	60	52	48	36	24
4.....	..	....	....	..	..	2.9	+	9.6	60	60	50	42	30

* Intermittent obstruction.

The technic of the bromsulphalein test in dogs was the same as that used previously with phenoltetrachlorphthalein. The dye was injected intravenously in a dose of 10 mg. for each kilogram of body weight, twice the dosage used for patients. In thirty determinations on twenty-two normal animals we found that the greater part of the dye disappeared within fifteen minutes. The usual readings of the percentage of dye present in the serum were between 10 and 22 after five minutes with an average of 17; between 3 and 14 after ten minutes, averaging 8; and 6 or less after fifteen minutes. Only traces of dye were found

5. Rosenthal, S. M., and White E. C. (footnote 3, second reference).

in the specimens of blood drawn an hour after its administration. We have compared the results of the different tests on the basis of the reading obtained fifteen minutes after the injection of the dye.

The changes in the bromsulphalein test were qualitatively the same as those previously reported in the phenoltetrachlorphthalein test. Retention of bromsulphalein in the blood stream of the dogs was not observed until the second or third day following ligation of the common bile duct. The development of distinct retention usually coincided with the first definite appearance of bilirubin in the blood, both occurring from forty-eight to seventy-two hours after operation. The

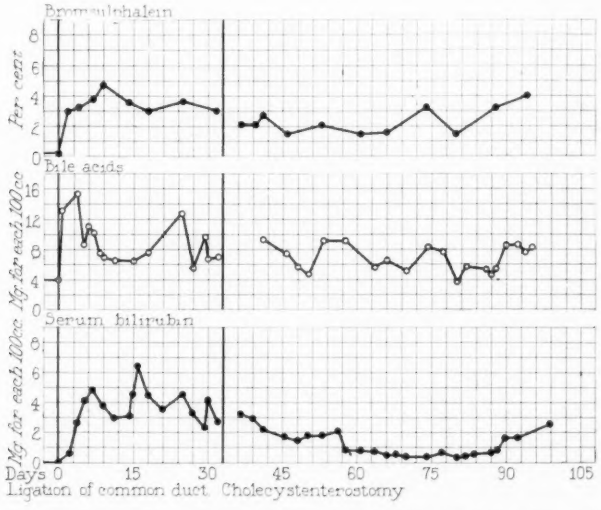

Fig. 5 (experiment 4).—Changes in bromsulphalein test, bile acid reading and serum bilirubin following ligation of the common bile duct. Cholecystoduodenostomy was performed on the thirty-third day after obstruction.

amount of retention gradually increased, the maximal value being reached the second week. Thereafter the degree to which the bromsulphalein was retained in the blood stream fluctuated somewhat, but in general there was marked and persistent retention of the dye.

When the gallbladder was removed at the time of the ligation of the common bile duct, retention of the dye was found within twenty-four hours after the operation. Here, too, a close parallelism with the degree of retention of bile was observed. The subsequent course of the two series of animals was identical. Retention of the dye persisted following cholecystenterostomy and the reestablishment of biliary drainage.

Bile Acids.—The bile acids in the blood were determined by the quantitative Pettenkofer reaction developed by Aldrich.[6] The results are reported in terms of milligrams of glycocholic acid for each hundred cubic centimeters of whole blood. The bile acids in the blood, so determined, increased markedly after the production of biliary obstruction. This increase was not marked until the second or third day following ligation of the common bile duct alone. Maximal values were attained about the second week after obstruction. When the gallbladder was removed at the time the common duct was ligated, the changes in the bile acid reading developed much more rapidly. The increase was marked within the first hour. The amount of retention in the blood gradually became greater, maximal values being attained at the end of the first week. Thereafter there was a gradual return toward normal. In experiment 1, for example, the bile acids returned to the normal value after fifty days of obstruction. They remained at approximately the normal level thereafter, although the readings were much more variable than in a normal dog. In experiment 2, normal readings were obtained the fifth week. Thereafter considerable fluctuation was observed, although the increases were never so marked as during the week following obstruction.

In experiment 4 the initial rise in the Pettenkofer reading reached a level of only 15.5 mg.; then there was a rapid fall. Relief of the obstruction had but little effect, and the values afterward fluctuated between 3.6 and 9.6 mg. In experiment 3, on the other hand, higher values were obtained after the obstruction. There was an increase to 30.8 mg. the day before the cholecystenterostomy and a rapid fall to 14.2 mg. the day following. The bile acid readings in this dog remained continuously elevated even though the biliary flow was partially reestablished. Here, too, the quantities in the blood showed great fluctuation.

Elimination of Bile Acids.—We have shown elsewhere the rapidity with which bile acids are removed from the blood stream after their timed intravenous injection.[7] In experiment 4 the dog was given such an injection of 200 mg. for each kilogram each hour before the ligation of the common duct. This experiment was repeated after the obstruction was established. The comparison of the changes in the blood in the two experiments (fig. 6) shows not only that the bile acids in the blood were more markedly increased by this measure, but also that

6. Aldrich, Martha: Studies in the Metabolism of the Bile Acids: I. A Quantitative Pettenkofer Test Applicable to the Determination of Bile Acids in the Blood, J. Biol. Chem., in press.

7. Snell, A. M., and Greene, C. H.: Studies in the Metabolism of Bile Acids: III. The Rate of Elimination of Bile and Its Constituents From the Blood After Their Intravenous Injection, in press.

they were removed at a much slower rate than in the normal. Such a change suggested the possibility of following the curve of elimination of the bile acids as a measure of the functional activity of the liver. Commercial preparations of bile salts were used. These were assayed and given intravenously in a dosage equivalent to 125 mg. of glycocholic acid for each kilogram of body weight.[8] The solution was rapidly injected. Samples of blood were then taken, five, fifteen, thirty, forty-five, sixty, and one hundred twenty minutes after the injection.

In normal animals the injected bile acids are rapidly removed from the blood stream. Five minutes after injection the amount varied between 32 and 46 mg.; at thirty minutes, between 10 and 23 mg.; the normal value for the Pettenkofer reading was regained by the end of the hour.

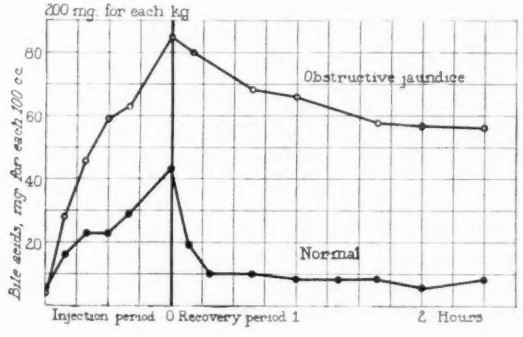

Fig. 6.—Rate of elimination of bile acids from the blood after their timed intravenous injection. The normal curve and that obtained after the experimental production of obstructive jaundice are shown.

Following the production of biliary obstruction, there was increasing difficulty in the elimination of the injected bile acids from the blood stream. At first (table 5) the bile acids failed of elimination within the usual time, and high readings were obtained an hour and two hours after the injection. Later, not only was there great delay in excretion, but the whole level of the curve was also increased to nearly double the initial reading: in one instance the amount in the blood five minutes after injection was 80.5 mg., and two hours later, 37.5 mg. This is shown graphically in figure 7, which presents the changes in another animal.

8. Bledsoe, Mary Sue; Aldrich, Martha, and Greene, C. H.: Studies in the Metabolism of the Bile Acids: II. The Estimation of Bile Acids in the Bile, J. Biol. Chem., in press.

Although injections were made every second or third day, there was no evidence of an accumulation of bile acids within the body. The quantities injected were apparently either eliminated through the urine or else destroyed. The experiments of Brakefield and Schmidt [9] on the changes in the urine following biliary obstruction demonstrate the urinary excretion of injected bile acids, and so support the former view.

COMMENT

The changes observed in obstructive jaundice in this series of experiments confirm and extend observations made in our earlier paper. The decrease in the blood urea, especially in relation to the nonprotein nitrogen in the blood, is definite although not marked.

TABLE 5.—*Changes Observed Following Ligation of the Common Bile Duct* *

Date	Time After Obstruction, Days	Hemoglobin, Gm.	Nonprotein Nitrogen, Mg., Per Cent	Blood Urea, Mg., Per Cent	Serum Bilirubin, Mg., Per Cent	Van den Bergh Direct Reaction	Bromsulphalein Test: Dye in Serum, Per Cent: 5 Minutes	10 Minutes	15 Minutes	1 Hour	2 Hours	Elimination of Bile Salt, Time After Injection: Control	5 Minutes	15 Minutes	30 Minutes	1 Hour	2 Hours
1926												Mg. for each 100 cc.					
11-18...	0	....	21	28	0	0	6	2	2	0	0						
11-19...	0	14.7	32	28	..	..	..	..	..	..	..	6.6	36.3	26.2	10.2	6.5	6.1
11-23...	Ligation of common bile duct																
11-24...	1	13.7	24	8	0	0	30	22	22	22	14	....	....	....	....	....	....
11-25...	2	....	..	..	..	..	..	..	..	..	..	8.6	41.1	31.2	27.0	27.0	23.4
11-26...	3	8.9	33	22	1.8	+	40	30	30	20	12	....	....	....	....	....	....
11-27...	4	....	..	..	....	..	..	..	..	..	..	11.5	65.6	60.0	54.3	45.1	27.8
11-29...	6	12.0	41	16	2.3	+	60	40	30	20	15	....	....	....	....	....	....
11-30...	7	....	..	..	....	..	..	..	..	..	..	9.7	80.5	51.9	48.4	38.2	37.5
12-3....	11	13.2	34	16	3.7	+	60	35	35	16	12	....	....	....	....	....	....
12-4....	12	....	..	..	....	..	..	..	..	..	..	15.6	71.5	62.1	53.4	43.2	25.8
12-6....	14	....	..	..	....	..	..	..	..	..	..	11.0	63.0	47.3	38.8	24.4	29.6
12-8....	16	....	35	17	2.8	+	60	30	22	12	10	....	....	....	....	....	....

*Experiment not described.

The changes in the serum bilirubin during the first few days after obstruction of the common bile duct are determined largely by the presence or absence of the gallbladder, as recently stressed by Mann and Bollman.[4] We were able to keep some of these dogs with complete biliary obstruction alive and in good condition for a considerable period. After the first few weeks, such animals showed a gradual decrease and almost complete disappearance of the jaundice. At the same time, there was a gradual reduction in the serum bilirubin to a level of from 1 to 3 mg. This reduction is not due to increased renal elimination of pigment, for Brakefield and Schmidt [9] have shown the

9. Brakefield, J. L., and Schmidt, C. L. A.: Studies on the Synthesis and Elimination of Certain Bile Components in Obstructive Jaundice, J. Biol. Chem. **67**:523, 1926.

gradual diminution in the urinary output of bilirubin in obstructive jaundice. A similar condition is observed in patients. In cases of obstructive jaundice of short duration, as from carcinoma of the head of the pancreas, higher serum bilirubin values occur, as a rule, than in cases of long-standing obstruction, as from stone in the common duct. There are numerous exceptions, but both the clinical and experimental evidence suggests that the production of bilirubin is decreased in consequence of prolonged biliary obstruction. In some cases this may be related to the reduction in the hemoglobin, as suggested by Rous and Drury.[10] On the other hand, we do not feel that the hemoglobin content of the blood is necessarily an accurate index to the degree of physjologic wastage of corpuscles, or the sole controlling factor in pigment metabolism.

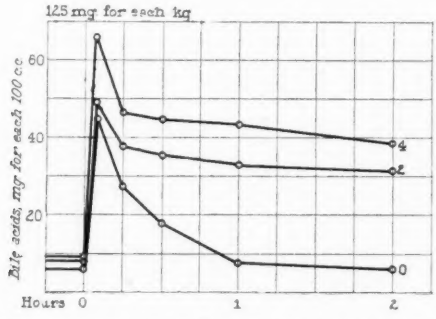

Fig. 7.—Change in the rate of elimination of bile acids from the blood during the development of obstructive jaundice. The bile salt solution was injected rapidly. The figures indicate time in days after ligation of the common bile duct and removal of the gallbladder.

The changes in the bromsulphalein test conform accurately to those previously obtained by the use of phenoltetrachlorphthalein. In each case retention of dye developed to an extent apparently conditioned by the rate of development and degree of the retention of the bile. With long-standing obstruction, retention of dye was relatively more marked and did not decrease as did the bilirubin level in the serum. The injection of bromsulphalein does not produce thrombosis of the vein as readily as does phenoltetrachlorphthalein. It also seems to be somewhat more sensitive to slight changes in the liver; hence bromsulphalein is preferable for routine clinical use.

The identification of bile acids in the blood on the basis of a colorimetric test such as the Pettenkofer reaction is open to criticism because

10. Rous, P., and Drury, D. R.: Jaundice as an Expression of the Physiological Wastage of Corpuscles, J. Exper. Med. **41**:601, 1925.

this reaction is not wholly specific. The presence of bile acids in the blood in obstructive jaundice has been accepted since the early experiments of Kühne [11] and Huppert.[12] More recently Perlzweig and Barron [13] reported positive colorimetric tests for bile acids in the blood of patients with obstructive jaundice, while Rosenthal and Wislicki [14] have obtained similar results with a modification of the amino-acid determination of Foster and Hooper. These results, together with the accepted fact of the urinary excretion of bile acids in this condition, furnish sufficiently direct evidence to justify our assumption that, in obstructive jaundice, the changes in the Pettenkofer reaction may be used as an index to changes in the amount of bile acids in the blood.

The bile acids in the blood, as determined by this method, increase temporarily after the production of biliary obstruction. The amount in the blood may be increased for two weeks, but it then decreases, and the normal values for the blood are gradually regained. Malkoff,[15] and Brakefield and Schmidt [9] have demonstrated that the curve of urinary excretion of the bile acids in obstructive jaundice shows similar changes. They point out that in this condition injected bile acids are largely, though not quantitatively, excreted in the urine. They further point out that the urinary output of bile acids in animals with obstructive jaundice is only a small fraction of the normal or of that which a dog with a biliary fistula can synthesize. From this they conclude that as a result of biliary obstruction there is a decreased synthesis rather than an increased breakdown of taurocholic acid.

Our experiments are in agreement with this view. The rapidity with which injected bile acids are removed from the blood stream of normal animals will explain their failure to appear in the urine in the control experiments of Brakefield and Schmidt. Biliary obstruction and stasis promptly blocks the normal pathway of excretion, and injected bile acids then leave the blood at a much slower rate than normally. In these experiments the amount injected was too small to cause permanent retention. If this was not completely excreted by the kidneys, the body was presumably able to destroy the excess. It should be noted, however, that the injected bile salts were not without toxic

11. Kühne, W.: Beiträge zur Lehre vom Icterus. Eine physiologisch-Chemische Untersuchung, Arch. f. path. Anat. u. Physiol. **14**:310, 1858.

12. Huppert, H.: Ueber das Schicksal der Gallensäuren im Ikterus, Arch. f. Heilkund. **5**:236, 1864.

13. Perlzweig, W. A., and Barron, E. G.: New Colorimetric Method for the Determination of Bile Acids in Blood, Proc. Soc. Exper. Biol. & Med. **24**:233, 1926.

14. Rosenthal, F., and Wislicki, L.: Ueber eine quantitative Bestimmung der Gallensäuren im Blute, Arch. f. exper. Path. u. Pharmakol. **127**:8, 1926.

15. Malkoff, G.: Zur Pathologie des Ikterus. Ueber die Ausscheidung der Gallensäuren durch den Harn, die Bauchwassersucht und einige andere Erscheinungen bei der Gallenretention, Jahresb. ü. d. Fortschr. d. Thierchem. **27**:785, 1897.

action, for animals so treated were not in as good condition and did not live as long as the other dogs in this series.

Reference should also be made to the biliary cirrhosis which developed in all the dogs that survived for more than a few weeks. The morphologic changes are too well known for especial comment. The functional changes recorded serve to explain some of the difficulties with cholecystenterostomy in these animals. In two dogs an attempt was made to relieve the biliary obstruction on the thirtieth and fortieth day, respectively. In neither did the bromsulphalein test show improvement following cholecystenterostomy. The serum bilirubin decreased a little, but the change was slightly, if any, more rapid than in those animals in which the obstruction persisted. The bile acids rapidly return toward normal after relief of the obstruction. This too may be related to the spontaneous decrease observed in all animals. The extremely rapid fluctuations in the bile-acid level observed in experiment 3, in which there was a period of intermittent obstruction, suggest that they are rapidly removed from the blood with the reestablishment of the biliary flow. Such an observation would be in harmony with the recognized normal rapidity of excretion of injected bile salts.

The development of ascites, collateral circulation and other evidences of portal obstruction which occurred in two animals in our series, as in the dog reported by Malkoff, is of much clinical and experimental interest. The ascites seen in such long-standing obstructive biliary cirrhosis is related to the widespread proliferation of connective tissue around the biliary radicals in the portal spaces, with consequent obstruction to the portal venous return. Malkoff further postulates changes in the blood as an additional factor predisposing to ascites. Mangelsdorf,[16] Ford,[17] and Weber,[18] have described the development of ascites in the terminal stages of biliary cirrhosis in consequence of continuous and prolonged biliary obstruction in man, and we have seen such cases. These animal experiments furnish the counterpart to this clinical condition.

SUMMARY

The effect of ligation of the common bile duct in dogs and the consequent experimental production of obstructive jaundice have been studied in more detail than was possible at the time of our original report. It was possible to keep two dogs with complete biliary obstruction alive for more than three months. In these dogs an extensive collateral circulation and ascites developed, thus providing an experimental

16. Mangelsdorf, J.: Ueber biliäre Lebercirrhose, Deutsches Arch. f. klin. Med. **31**:522, 1882.

17. Ford, W. W.: Obstructive Biliary Cirrhosis, Am. J. M. Sc. **121**:60, 1901.

18. Weber, F. P.: On Biliary Cirrhosis of the Liver With and Without Cholelithiasis, Tr. Path. Soc. London **54**:103, 1903.

counterpart of the advanced biliary cirrhosis seen clinically in association with prolonged biliary obstruction.

During the period immediately following ligation of the common duct, the changes in the blood urea, serum bilirubin and the degree of retention of dye were similar to those previously reported. The effect of the presence or absence of the gallbladder in determining such changes was shown to hold, not only for the bilirubin but also for the bromsulphalein test and for the bile acids in the blood.

The bilirubin content of the serum tends to decrease spontaneously in the later stages of obstructive jaundice, presumably due to a decrease in the production of pigment. With the development of cirrhotic changes in the liver the excretion of bilirubin apparently becomes increasingly difficult, and in two dogs cholecystenterostomy with temporary relief of the biliary obstruction did not significantly affect the level of bilirubin.

The changes in the bromsulphalein test were in entire agreement with those previously obtained with phenoltetrachlorphthalein. The retention of dye in its development closely paralleled the degree of retention of bile. Once retention was established, the bromsulphalein readings were relatively constant and its retention more persistent than that of either the bile acids or the bilirubin.

The intensity of the Pettenkofer reaction given by the blood increased rapidly after obstruction. The rate of accumulation of bile acids under these circumstances was determined by the activity of the gallbladder, as in the case of the bilirubin. Later the readings tended to decrease, and returned approximately to the normal value, indicating probable failure of the synthesis of the bile acids. The changes in the blood corresponded with the reported changes in the urinary excretion of the bile acids. We believe, therefore, that the changes in the Pettenkofer readings may be considered indicative of changes in the bile acid content of the blood.

Intravenously injected bile acids are rapidly removed from the blood stream of normal animals. This elimination was interfered with in obstructive jaundice, and injected bile acids were retained in the blood stream, the degree of retention paralleling the changes in the other tests.

These experimental data are a counterpart of, and in agreement with, the changes observed clinically in obstructive jaundice and in biliary cirrhosis secondary to obstruction of the bile passages.

NORMAL VARIATIONS IN WHITE BLOOD CELLS UNDER CONDITIONS OF MINIMAL METABOLISM *

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The white blood cells of normal persons have been the subject of many and varied forms of investigation since they were first described in 1777 by Hewson.[1] As a consequence, limits of normal have been established both for the total numbers per cubic millimeter of circulating blood and for the percentages of the types of cells comprising this total. These normal limits have been determined from observations made once on each of a group of healthy persons or from data obtained by repeated observations of the same person's blood over a period of hours. The influence of various physiologic processes on the white blood cell picture has also been studied extensively. However, comparatively little information is available concerning the fluctuations of the nucleated blood cells that may occur over an extended period of time in a normal person. Such observations as have been made appear not to have been conducted under conditions of minimal metabolism.

The fluctuations occurring during the day have been repeatedly studied. Turk [2] considers, as do others, that the white blood cell count is at a minimum in the early morning, when the person is at complete rest and before the ingestion of food. Then it may be below 5,000 cells per cubic millimeter, with a marked depression of polymorphonuclear neutrophils and a relative abundance of lymphocytes, often amounting to from 30 to 40 per cent of the total and sometimes more. Mauriac and Cabonat [3] also found that the greatest constancy in the absolute and differential white blood cell count occurred in the early morning. Like others, they record a marked fluctuation during the day. Sabin and her

* From the Medical Service of the Collis P. Huntington Memorial Hospital of Harvard University. This work was aided by a grant from the Proctor Fund of the Medical School of Harvard University for the study of chronic disease.

1. Hewson, William: A Description of the Particles of the Blood in the Human Subject and in Other Animals with an Account of the Structure and Offices of the Lymphatic Glands, of the Thymus Gland, and of the Spleen, London, 1777. From the Works of William Hewson, F. R. S., edited by George Gulliver, Sydenham Society, 1846, p. 251.

2. Turk, W.: Vorlesungen über klinische Hämatologie, Wien, W. Braumuller, 1912, vol. 2.

3. Mauriac, P., and Cabonat, P.: Contribution à l'étude des variations de la formule leucocytoire chez l'homme normal, Paris méd. **11**:407 (May 21) 1921.

associates,[4] in a systematic study of several persons over periods of hours, have demonstrated a characteristic and approximately hourly rhythm of the circulating white blood cells which is independent of digestion or exercise. They found that the total white blood cell count might vary as much as 100 per cent in the course of the day. The higher values were obtained in the afternoon and were largely due to increases in the polymorphonuclear neutrophils. Other types of cells showed characteristic, although less extreme, rhythmic fluctuations. Somewhat similar observations have been recorded by other investigators.[5]

Appreciable variations in the absolute numbers of white blood cells have been noted in counts of blood taken at approximately the same hour on successive days. Spiethoff[6] and Michailow[7] reported that this variation was frequently as great as 2,000 cells per cubic millimeter. They found, however, that the percentage relationships of the various types of cells were remarkably constant and that no reciprocal relationship occurred between the polymorphonuclear neutrophils and lymphocytes. Heintz and Welker,[8] on the other hand, showed a greater constancy in the daily total white blood cell count of twenty-two normal subjects whose blood was examined at approximately the same hour each morning soon after breakfast for a seven-day period. Their counts demonstrated an average daily fluctuation of only about 300 cells per cubic millimeter and a maximum difference of 1,600 per cubic millimeter in any one subject during the entire period of observation. These variations are hardly beyond those which may be accounted for by probable error.

4. Sabin, F. R., Cunningham, R. S., Doan, C. A., and Kindwall, J. A.: The Normal Rhythm of the White Blood Cells, Bull. Johns Hopkins Hosp. **37**:14 (July) 1925.

5. Spiethoff, B.: Besteht nach die Kriegslymphocytose, München. med. Wchnschr. **69**:1532 (Nov. 3) 1922; Zur Methode der Blutuntersuchungen und Mitteilungen über fortlaufende Blutuntersuchungen an Gesunden, Folia haemat. **32**:325 (July) 1926. Kobryner, A.: La leucocytose physiologique chez l'homme, Compt. rend. Soc. de biol. **90**:1475 (June 6) 1924; Uber den physiologischen Verlauf der Leukozytose beim Menschen, Deutsche med. Wchnschr. **50**:1218 (Sept. 5) 1924; Physiologic Course of Leukocytosis in Man, Ztschr. f. klin. Med. **102**:470, 1925. Glaser, F., and Buschmann, P.: Die Bedeutung der Spontanschwankungen der Leukozyten (besonders für die hämoklasische Krise un die Verdauungsleukozytose), Med. Klin. **19**:1144, 1923. Jimenez Diaz, C.; Espejo, M., and De Avellaneda, G.: Leukocyte Variation Curve and its Significance, Arch. de Cardiol. y Hematol. **6**:1118, 1925. Woronoff, A., and Riskin, J.: Ueber die Leukozytose bei normalen Menschen und Hunden, Wien. Arch. f. inn. Med. **10**:45 (April) 1925.

6. Spiethoff: Footnote 5, second reference.

7. Michailow, F.: Die Schwankungen der Leukozytenzahl und Leukozytenformel im peripherischen Blute des Menschen und die Verdauungsleukozytose, Folia haemat. **32**:196 (May) 1926.

8. Heintz, E. L., and Welker, W. H.: The Effect of the Ingestion of Yeast on the Leukocyte Count, Arch. Int. Med. **35**:500 (April) 1925.

It is evident that the white blood cell formula is by no means a fixed ratio, but may be subject to appreciable changes during the day and from day to day even under standard conditions. In evaluating counts of the blood cells, one must appreciate not only the degree and kind of physiologic variations and the technical error of the methods, but also the mathematical error arising from the numbers of cells counted. This has been fully discussed recently by Brandt,[9] who allows a range of about 30 per cent in the total white blood cell count as being the limit of "fortuitous error." Consistency in repeated observations must be considered significant, however, even in the absence of absolute mathematical certainty.

The present study was undertaken to observe any degree of constancy that might be obtained over an extended period of time in the daily total and differential white blood cell count of normal persons under conditions of minimal metabolism.

MATERIAL AND TECHNIC

The subjects were six healthy medical students in the third decade of life, who were living hygienically under comparative conditions. Their blood was obtained in the morning before they got out of bed, except as noted below. The blood of two (A and B) was examined approximately every other day for six weeks in the late autumn; that of the others (C, D, E and F) was examined every day for ten days during the month of February. Every other day for periods of weeks at different times of the year, smears were also made of A's blood immediately after he had arisen.

The blood for the white blood cell counts and cover glass smears was taken from the finger of A and from the lobe of the ear of the other persons. A minimum amount of manipulation was employed in obtaining the blood, and the same pipet and hemocytometer were used in counting each person's blood. Careful attention was given to following the recognized technic for an accurate count of the corpuscles and the preparation of evenly spread films of blood. All enumerations were made by the same person. Never less than 200 cells, usually 400 and sometimes more, were counted from the two coverslips made at one time to determine the percentages of the different types of cells. Five classes of cells were recorded: polymorphonuclear neutrophils, eosinophils, basophils, lymphocytes (small, large and atypical) and monocytes. Fragmented forms were not included. The rare observations of certain immature forms were listed separately.

9. Brandt, T.: Ueber die Fehlerberechnung der hämatologischen Methoden; ein Beitrag zur kritischen Beurteilung der gefundenen, Werte, Folia haemat. **32**:177 (May) 1926.

Tables 1 and 2 summarize the results of the white blood cell counts on the six students under conditions of minimal metabolism.

Charts 1 and 2 illustrate graphically the variations in the differential and absolute enumerations of the white blood cells of student D. The graphs for D are entirely comparable to those for the other subjects, although some showed slightly more and some less daily variation in the counts.

TABLE 1.—*Ten to Fourteen Daily White Blood Cell Counts of Six Normal Persons Under Conditions of Minimal Metabolism*

Individual	White Blood Cells (*)	Polymorphonuclear Neutrophils (†)		Lymphocytes (‡)		Monocytes	
	Per Cu. Mm.	Per Cu. Mm.	Per Cent	Per Cu. Mm.	Per Cent	Per Cu. Mm.	Per Cent
A Av.	6,700	3,690	53.6	2,219	33.7	618	9.3
Min.	5,000	2,421	46.5	1,842	29.0	332	5.0
Max.	7,700	4,750	61.5	2,584	39.0	840	12.0
B Av.	5,832	3,034	51.4	2,183	37.0	459	7.8
Min.	4,800	2,290	45.0	1,765	29.5	234	4.5
Max.	7,150	3,680	58.5	2,860	45.0	793	12.0
C Av.	5,547	2,845	51.3	1,838	33.2	630	11.3
Min.	4,975	2,310	40.5	1,477	27.2	277	5.5
Max.	6,450	3,775	62.7	2,306	40.7	908	14.7
D Av.	7,425	3,726	50.3	2,706	36.4	781	10.5
Min.	5,425	2,760	44.0	1,860	29.7	502	8.5
Max.	9,550	5,110	57.7	4,020	44.0	1,048	12.2
E Av.	4,987	2,723	54.7	1,662	33.3	500	10.0
Min.	4,050	2,154	50.5	1,293	30.2	360	8.0
Max.	7,150	3,510	58.2	2,573	36.5	786	12.0
F Av.	7,632	3,451	45.3	3,192	41.8	729	9.5
Min.	6,800	2,975	39.7	2,312	33.5	527	7.7
Max.	9,025	4,075	55.0	3,801	48.0	930	11.0

* While the maximum variation in no case occurred between consecutive counts, there was frequently a variation of 1,800 cells per cubic millimeter from one day to the next.

† For purposes of condensation, the values for polymorphonuclear eosinophils and basophils have been omitted from this table. The former varied between 0 and 5.2 per cent, the latter between 0 and 3.5 per cent. The averages are shown in table 2.

‡ The large lymphocytes usually formed about 4 per cent of all the white blood cells.

TABLE 2.—*Averages and Extremes of All Counts (62) on All Six Persons*

	White Blood Cells	Polymorphonuclear, Neutrophils		Eosinophils		Basophils		Lymphocytes		Monocytes	
	Per Cu. Mm.	Per Cu. Mm.	Per Cent	Per Cu. Mm.	Per Cent	Per Cu. Mm.	Per Cent	Per Cu. Mm.	Per Cent	Per Cu. Mm.	Per Cent
Av...	6,309	3,216	51.04	148	2.31	55	0.85	2,294	36.04	609	9.62
Min..	4,050	2,154	39.75	00	0.00	00	0.00	1,293	27.25	234	4.50
Max.	9,550	5,110	62.75	407	5.25	241	3.50	4,020	48.00	1,048	14.75

Variations in the total white blood cell counts from day to day were often greater than 1,000 and even as great as 2,400 cells per cubic millimeter. The extreme variation shown by any one person during the entire period of observation was 4,125 cells per cubic millimeter, while the least maximum variation was 1,475. A variation of 1,000 per cubic millimeter in the number of white blood cells when the total number is about 7,000 is not mathematically significant. However, variations occurred greater than the mathematical error, and the constant fluctuation from day to day cannot be explained as due to technical

or mathematical errors. The degree of these fluctuations in the numbers of white blood cells under conditions of minimal metabolism is not as great as has been observed in relatively inactive persons throughout the day.

The fluctuations in the total number of white blood cells was not dependent on any one type of cell, although the polymorphonuclear neutrophils, perhaps owing to their greater frequency, exerted the most apparent effect. The percentage of polymorphonuclear neutrophils was found to be considerably lower than the commonly accredited figures (about 60 per cent) for normal persons in this locality. The values

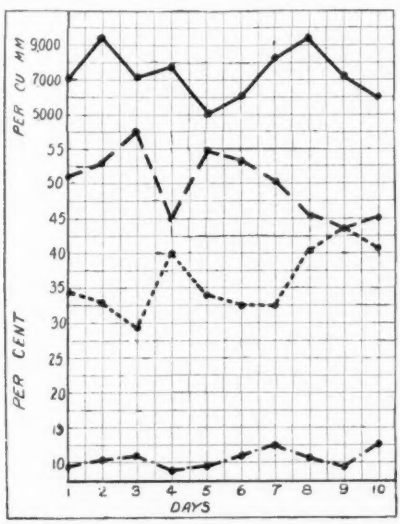

Chart 1.—Daily variations in the percentages of white blood cells in a normal individual (D) under conditions of minimal metabolism. The solid line represents the total of white blood cells per cubic millimeter; the broken line represents the percentage of polymorphonuclear neutrophils; the dotted line, the percentage of lymphocytes, and the dot and dash line, the percentage of monocytes.

ranged from 39.7 to 62.7 per cent, the average for all counts being 51.04 per cent. If a deviation of 5 per cent is allowed on either side of this average as due to "fortuitous error," many of the variations shown by these subjects are to be interpreted as an actual change in the number of cells. The same is true for the fluctuating percentage of lymphocytes, which ranged from 27.2 to 48 per cent, the average being 36.04 per cent. The general trend of these figures is in accordance with those obtained by Turk [2] and others, who have observed that before breakfast there is a smaller percentage of polymorphonuclear elements and a

greater percentage of lymphocytes in the circulating blood than later in the day.

The fluctuations that occurred among the other types of normal white blood cells were slight and require no comment. Myelocytes have been observed occasionally in the blood of normal persons as, for example, by Sabin [4] and her co-workers. Such cells were found infrequently in this series of observations. Atypical cells, apparently of lymphoid origin, were encountered on five occasions.

Chart 3, besides illustrating the daily fluctuations that occurred in the differential white blood cell formula of one person (A), also shows

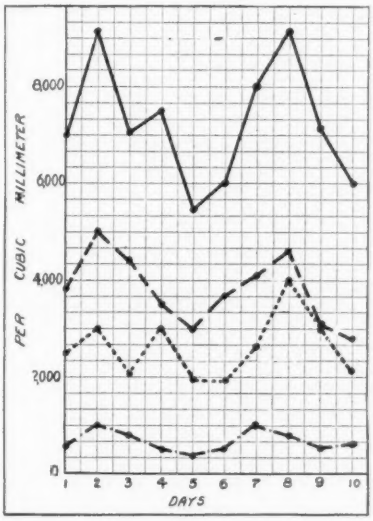

Chart 2.—Daily variations in the absolute numbers of white blood cells per cubic millimeter under conditions of minimal metabolism. The percentage values of the same counts are recorded in figure 1. The continuous line in this chart represents the total of white blood cells per cubic millimeter; the broken line, the polymorphonuclear neutrophils per cubic millimeter; the dotted line, the lymphocytes per cubic millimeter, and the dot and dash line, monocytes per cubic millimeter.

the definite change observed in the relative frequency of polymorphonuclear neutrophils and lymphocytes over a period of months. There was a slight, but consistent decrease in the percentage of polymorphonuclear neutrophils with a relative increase of lymphocytes for a period of time in the autumn and early winter as compared with the values obtained in the late spring, summer and midwinter. The period showing the highest average percentage (63.24) of polymorphonuclear neutrophils was in June. At that time, the lymphocytes averaged 25.86 per

cent. The lowest average percentage (47.8) of neutrophils occurred in December when the lymphocytes averaged 38.67 per cent. This difference in values appears to be of sufficient magnitude to be mathematically significant.

Exposure to the sun's rays induces an increase of lymphocytes.[10] This can account for the increase in lymphocytes observed by Taylor [11] in twenty-five of the thirty-eight persons studied by him before and after they had spent a summer at Woods Hole, Mass. The increase of lymphocytes recorded in white people in the tropics or in the Philippines [12] may also be attributed partially to sunlight. Thus, the effect of sunlight may be a seasonal factor in influencing the differential white

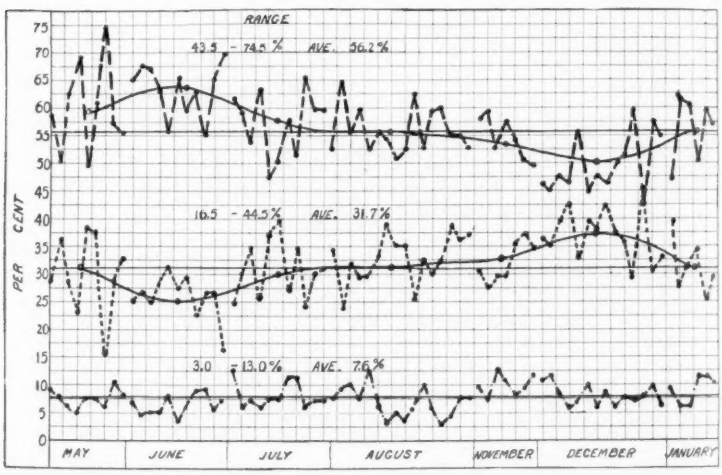

Chart 3.—Variations in the percentages of the white blood cells of one individual (A) under conditions of minimal metabolism over a period of months. The broken line represents the polymorphonuclear neutrophils; the dotted line, the lymphocytes; and the dot and dash line, the monocytes. The straight heavy lines represent the averages for the whole series of counts (74). The continuous curved lines connect the points representing the averages of the several periods.

blood cell count, but it seems unlikely that this is the factor responsible for the increase in lymphocytes noted during December in the observations recorded here. Alterations in the white blood cell formula during

10. Clark, J. H.: Action of Light on the Leukocyte Count, Am. J. Hyg. **1**:39 (Jan.) 1921. Aschenheim, E.: Der Einfluss der Sonnenstrahlen auf die leukocytäre Blutzusammensetzung, Ztschr. f. Kinderh. **9**:87, 1913.

11. Taylor, H. D.: The Effect of Exposure to the Sun on the Circulating Lymphocytes in Man, J. Exper. Med. **29**:41 (Jan.) 1919.

12. Wallace, J. B.: The Differential Leucocyte Count in a Sub-Tropical Climate, South. M. J. **17**:827 (Nov.) 1924. Wickline, W. A.: The Effects of Tropical Climate on the White Race, Mil. Surgeon **23**:282, 1908.

the various seasons of the year are of interest to speculate on, but the relatively scanty data at hand and the lack of other similar observations preclude drawing conclusions.

It is known that ingestion of lecithin will produce a transient lymphocytosis,[13] while a relatively high percentage of lymphocytes is not rare in obese persons.[14] The question thus arose as to whether a diet rich in fat could maintain the percentage of lymphocytes at a relatively high level. In an effort to answer this question, subject A took a daily diet consisting of fat, 190 Gm.; protein, 86 Gm., and carbohydrate, 200 Gm., for nine days in June and for five days in December. This diet contained approximately twice the amount of fat eaten during the other periods of observation. Although not deliberately chosen, it happened that the diet rich in fat was taken during the periods when the average of lymphocytes was at a low level (June) and at a high level (December). However, the diet as taken did not result in any increase of lymphocytes attributable to the fat. This negative observation by no means precludes the possibility that a diet rich in fat taken for a long period of time may induce an increase of lymphocytes in certain people.

SUMMARY

The white blood cells of six normal persons under conditions of minimal metabolism were studied daily for ten days and the white blood cells of one of these persons were observed frequently in each of several months of the year.

The following conclusions are reached:

1. The polymorphonuclear neutrophils were fewer and the lymphocytes greater than usual during the day. The average percentage of the former was 51.04 and of the latter, 36.04.

2. Daily variations in the white blood cell count were beyond the limits dependent on technical or mathematical errors. Each type of cell shared in the fluctuations.

3. The average percentage of polymorphonuclear neutrophils and lymphocytes maintained different levels during different periods of the year.

4. Immature cells of both myeloid and lymphoid origin were encountered rarely.

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13. Bain, William: The Pharmacology and Therapeutics of Lecithin and Phytin, Lancet **1**:918 (April 6) 1912.

14. Caro, L.: Blutbefunde bei Adipositas, Berl. klin. Wchnschr. **49**:1881 (Sept. 30) 1912.

NATURAL MINERAL WATERS IN THE LIGHT OF MODERN RESEARCH

THE CATALYTIC ACTION OF THE SARATOGA SPRINGS *

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This paper deals chiefly with a report of chemical and physicochemical observations on mineral springs. We shall attempt to demonstrate that, through the discovery of new methods, the study of mineral springs has entered a new epoch. Long before the development of quantitative chemical analysis, mineral springs were empirically employed, with good results, in the treatment of many diseases. New aspects manifested themselves, however, when the chemist began to analyze the springs and to establish the nature and quantity of the salts contained in them. At first, naturally, chemical analysis was not well developed, and it was difficult, therefore, to demonstrate with certainty the presence of minute traces of elements. In this connection it is of interest to note that salt springs in Austria, which were traditionally known as "goiter-waters," appeared to contain only common salt until modern analytic chemistry showed that they also contained the therapeutically active iodine. There are many cases known in which man's instinct first found the remedy, while science, with its exact explanations, lagged behind. The classic example of this in balneology is the early recognition of curative springs which seemed to contain ordinary drinking water, but which later, after the discovery of radium, proved to be strongly radioactive.

SIGNIFICANCE OF TRACES OF ELEMENTS IN BIOLOGIC PROCESSES

In these examples we are confronted with the remarkable fact that the active substance of a curative water may be present in extremely small amounts, and that often only slight traces of a substance suffice to bring about profound biologic changes in animals and in man. Every thinking physician who would keep abreast of modern science must be conversant with the idea that amounts of substances which are hardly weighable and perhaps often only spectroscopically detectable are capable, under certain circumstances, of exerting great biologic effects and are, therefore, of therapeutic significance. In this respect, modern research on vitamin has taught us much that is astonishing.

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There is a broad and still virgin field in the investigation of the biologic action of the numerous substances in natural mineral waters, many of which are present only in traces. In the latter class is iron, an element widely distributed in nature. Iron often accumulates in considerable quantities in mineral springs; thus, the Moritz spring in Bad Elster, one of the strongest, iron-carbonic acid springs, contains 26 mg. of iron per liter.[1] The question of the rôle of iron in metabolic processes has acquired increasing importance in recent years. The metabolism of iron is more complicated than was formerly assumed, for contrary to other inorganic substances, resorbed iron is not carried directly to the site of its utilization, nor is waste iron directly eliminated. Stores of this element exist in the spleen, liver and bone marrow. In iron-free nutrition, serious anemia does not occur until from the second to the fourth generation. Long before the chemical determination of iron in mineral waters, these were successfully employed as remedies for anemia. The blood contains iron as an integral constituent, and by means of this element binds the oxygen of the air and transports it, apparently in mildly activated condition, to the sites of its utilization.

Respiration, which many physiologists designate, in concordance with Barnes, as "energesis," is, according to the new physiologic point of view, the most important of metabolic processes in the human organism. According to Warburg, iron is indispensable to respiration. Further, iron together with magnesium-containing chlorophyl is the transporter of solar energy in the reduction of carbonic acid by green plants—that fundamental, life-sustaining process of our planet. The oxidation of ferrous bicarbonate to ferric carbonate can serve as a source of energy for those lower plants which assimilate carbonic acid chemosynthetically.

These few examples should suffice to illustrate the great significance of iron in biologic processes. From a purely chemical and physical point of view also, iron is distinguished from all other elements particularly by its complex spectrum which signifies a large number of electron shifts, and further, by its special magnetic behavior. Of the many chemical peculiarities of iron, the one that interests us here above all is the catalytic properties of the metal, its salts and oxides. In our own research the catalytic properties of the iron dissolved in mineral waters were studied, and we shall report the results of our investigation in this article.

Before we describe our new experiments and discuss the significance of the constitution of a salt in relation to its catalytic and biologic properties, we shall first analyze, in a general way, the conditions or

1. Leidner, Tustus: Dissertation, Berlin, 1916. Bickel, Adolf; Gleidmann, F., and Taslakowa, T.: Ztschr. f. d. ges. exper. Med. **54**:87, 1927.

forms in which the individual constituents of so complex a salt mixture as a natural mineral water can theoretically come into play. The inorganic substances can occur in various states, and the following classifications may be used as a guide:

1. Ions (electrically charged atoms of the elements). 2. Complex-ions (in the Werner sense). 3. Undissociated molecules.[2] 4. Colloidal particles (unspecific surface action). 5. Submicroscopic crystals (specific surface action).

BIOLOGIC ACTION OF ELECTROLYTES AND ITS RELATION TO THE ACTION OF MINERAL WATER

An enormous amount of experimental work has been done on the relation between inorganic ions and healing action. This extends to the electrolytic colloid-chemical action of the salts dissolved in mineral waters. It is an interesting fact that for the optimum of life and its functions a definite ratio of sodium, potassium and calcium is necessary; from sea water to the blood of man this ratio is maintained at 100:2:2. A fundamental experiment on the significance of dissociated salts, the electrolytes, was made, as is well known, by Jacques Loeb, in which he showed that individual electrolytes were toxic to living organisms. Not only the sodium, calcium, magnesium and iron salts, however, but also the numerous rare electrolytes of the body take part in the metabolic mechanism. We still know too little of the action and significance of the many individual ions. It has been established, however, that the electrolytes play a part in determining the constitution of man, and that in the cooperation of many electrolytes is to be sought one of the fundamental curative factors in the therapeutic use of mineral springs. The use of drinking cures stands preeminent in the possibility that it offers of administering to the human body sufficient quantities of electrolytes in a dissociated state and in combination with many other ions. By means of baths, too, ions such as calcium can be brought through the skin, as Wermel has recently shown. Thus, for example the waters of Franzensbad and Nauheim, the world famous heart baths, contain, besides traces of iron, large quantities of calcium; they are supersaturated with carbonic acid and are also radioactive. The famous American springs at Saratoga are no less valuable, and they were chosen by us for experimental material.

COMPLEX COMPOUNDS AND WERNER'S THEORY

While, as has been briefly stated in the foregoing, we are relatively well orientated, as a result of rich experimental material, with regard to the physiologic action of the simple electrolytes in mineral waters,

2. For instance, as has been shown by Miss Marian Irwin, the dyestuff brilliant cresol blue penetrates into the living cells of Vitella in the form of the undissociated molecule (J. General Physiol. **9**:561, 1926).

our knowledge of the question of the formation and balneologic action of complex ions has not yet been analyzed. So complicated a salt mixture as natural mineral water, which also contains the complex-forming carbonic, silicic and boric acids, is predestined to form more or less stable complexes. The formation of complex ions stands in intimate relationship not only to resorption and assimilation, but also to the general biologic action of the physiologic metals. Before we go into the question of the existence of complex ferrous compounds in the Saratoga springs, we shall present a brief explanation of Werner's theory,[3] which, as is well known, forms the basis of inorganic structural chemistry. Werner's new conceptions of valence and his new interpretation of the stereochemistry of inorganic compounds will undoubtedly prove to be of the greatest value.

In order to explain Werner's ideas of valence in a few words, we shall choose as an example a typical so-called complex salt, namely, potassium ferrocyanide. In this salt $K_4Fe(CN)_6$, there are present, on one hand, linkages (between iron and cyanide radicles) which, when the salt passes from the crystalline state to the dissolved state, remain unchanged, and, on the other hand, linkages (between $[Fe(CN)_6]$, and the potassium atoms) which are ruptured by the action of the solvent with the formation of potassium ions and ferrocyanide ions $(Fe(CN)_6$ which undergo ionic migration as distinct individuals. These ions can recombine spontaneously with the restoration of the $K_4Fe(CN)_6$ lattice. We thus establish the existence of two types of linkage which differ markedly in their behavior. One type resists the change in state on going into solution, while the other type on going from the solid state into aqueous solution is broken down by the solvent, with the result that the individual ions from the ionic lattice come into evidence.

STRUCTURE OF INORGANIC COMPOUNDS AND THEIR RELATION TO BIOLOGIC PROCESSES

Since the introduction of the classic dissociation theory (1886), the biologic interest in ordinary inorganic salts has generally been limited to the action of the simple ions which are formed in solution. This limited point of view involves only the principal valencies and neglects entirely the auxiliary valencies which are exerted in the formation of complex compounds to which great biologic significance must be attached. In calcium chloride, for example, calcium and chlorine are joined by so-called principal valencies. Besides the principal valencies, however, the calcium possesses six auxiliary valencies, which are able to link atoms, molecules, groups or radicles, the calcium itself forming the

3. Werner, A.: Neuere Auschauungen auf dem Gebiete der anorganischen Chemie, Aufl. 4.

coordination center. Thus, to give only one example, the calcium in calcium chloride ($CaCl_2$) can still link coordinately four pyrocatechol and two methyl alcohol molecules, with the formation of the following complex salt.

$$Ca\left[\begin{matrix}(C_6H_4(OH)_2)_4\\(CH_3OH)_2\end{matrix}\right]Cl_2$$

A complex calcium phosphate forms the principal constituent of the mineral substance of bones and teeth. It has been shown only recently that the widely occurring mineral apatite [$CaCO_3.3Ca_3(PO_4)_2$] is identical with the calcium phosphate complex in the bones, not only in its chemical composition, but also in its submicroscopic structure (crystal architecture). In most mineral waters we find calcium carbonate besides small amounts of calcium phosphate, silicic acid and boric acid. We cannot consider mineral water, which contains so complex a mixture of salts, simply as a mosaic of the individual constituents, but must keep the idea before us that even liquids have a definite structure, in a certain sense; that is to say, affinities come into play between the substances in a solution and the water molecules thereof whereby inorganic molecules of definite structure are created. Water plants or sea animals are adapted to the structure of the liquid surrounding them in an exceedingly sensitive manner. In biologic fluids, moreover, the correlation between the dissolved substances is of greatest significance. They stand to each other in demonstrable physicochemical relationships, from which it results that the resorption of a substance from a solution is different, depending on whether it is present alone in solution or in company with others. Foods, too, act reciprocally; that is to say, each contributes to the assimilation of the other. Therefore, substances which are present only in small amounts become of the greatest importance because of these affinities, that is, by means of the formation of more or less stable complexes. Thus, for example, Spiro [4] has shown that the boric acid occurring in the water of Baden (Switzerland) is easily resorbed, because it is of itself lipoid-soluble and readily forms complexes the physiologic behavior of which deserves special attention.

We see from these examples that the physiologic action of a mineral spring cannot be judged from its chemical analysis. The recognition of this fact, however, does not by any means exhaust the biologic variability of a salt mixture. Fresh mineral water is crystal clear; in contact with air, however, a precipitate of originally dissolved material sooner or later separates from it. Well known examples are the precipitation of aragonite from the hot Carlsbad springs and of iron

4. Spiro: Schweiz. med. Wchnschr. **45**:1021, 1926.

compounds from iron-containing springs. We speak, in general, of the aging of a spring. Saratoga water, for example, becomes opalescent in contact with air in the course of minutes, and thereby loses important catalytic properties concerning which we shall report in detail later.

It is seen that by the contact with air and with the escape of carbon dioxide and other gases from the clear solution, a solid substance separates itself from the water. It is only in exceptional cases that nature produces a solid substance without at the same time giving it a specific form, a structure, which on its own part influences the chemical behavior of the substance. Consequently, we must direct our attention toward the formation (building up of the structure) of a solid substance as a result of its origination and transformation processes, as well as toward the mechanism by which its form serves in the course of chemical processes.

A definite process of formation leads to a definite structure, and the physiologic action of an inorganic salt depends, as we have found, much more on its constitution (inorganic structure) than on its chemical composition. Thus, one and the same substance (Fe_2O_3) in forms of different structures appears to act like two different chemical individuals.[5] In the formation of colloidal precipitates there are two factors, designated by Haber as arrangement and heaping velocities, which play an important rôle. These are not of invariable magnitude, but depend on the nature of the substance and on the external conditions. If we apply these relationships to the mineral water question, for example, to a spring that contains large amounts of dissolved calcium carbonate, then it will depend on the conditions mentioned whether or not the labile μ calcium carbonate first separates out and then goes over into the more stable form (aragonite). Although both carbonates have the same chemical composition, it is justifiable to assume, on the basis of the investigations of Welo and Baudisch, Webster and Baudisch, and of Bickel and his co-workers, that the physiologic action of the two can be different, depending on whether the one or the other is required for assimilation. This is true, first, because of the different solubilities and the closely related resorption and assimilation of the two substances; second, in consequence of the difference in the topography of the crystal surfaces and the closely related development of catalytically active atomic heaps (Taylor) or activated atoms; and third, in consequence of their different energy contents. Just as amorphous carbon, diamond and graphite have different energy contents which manifest themselves in the heat of combustion of these substances, so also must μ calcium carbonate (hexagonal), aragonite (rhombic), and calcite (rhombohedral) possess different energy con-

5. Welo, L. A., and Baudisch, O.: Science **52:** 311, 1925.

tents. In the one case, graphite presents the form which contains the least energy and is most stable, and in the other case it is calcite. It must be particularly noted here, not only that the three calcium carbonates evidence macroscopic crystallographic differences, but also that, according to Wyckoff,[6] the atoms of these substances possess different spatial arrangements and therefore different roentgen-ray spectrograms. Such facts seem to point out that in the modern investigation of the physiologic action of mineral springs and of inorganic salt mixtures in general, roentgen-ray spectroscopy is a new and not to be underestimated tool. By means of our roentgen-ray spectroscopic investigation of the so-called active and inactive iron oxides and their use as biologic catalyzers, the fact has been brought out that we must also consider the action of mineral salts biodynamically. In general, undoubtedly, the specific structure of inorganic salts and their changes in structure are bound up with life processes just as closely as is the structure of organic compounds.

In connection with the influence of light on simple inorganic compounds, we shall again speak of the Werner conception of valence, first setting down the following principles: We differentiate between electrovalence and coordination valence. In the former, we conceive that electrons of the outermost shell are transferred to the corresponding shell of another element. These are the valence electrons, and the forces involved are electrostatic—that is, we are dealing simply with the transfer of negative electricity. In coordination valence we know for a certainty today that not only purely electrostatic forces come into play, at least in the transition elements. The binding electrons which unite various radicles with a central atom do not lie on the outermost shell, but may come from deeper lying shells. Thus it happens that metallic atoms such as cobalt and nickel, which are similar with respect to their valence electrons and, therefore, with regard to their ordinary ionic reactions, have completely different properties in certain complex ions. The radicles bound to the central atom can often change the electronic architecture of the central atom most strikingly.[7]

DETERMINATION OF THE CATALYTIC ACTIVITY OF FRESH AND BOTTLED SARATOGA WATERS

In connection with the foregoing, we have been particularly interested in the catalase action of the pentacyano iron compounds. As will be seen from chart 1, the hydrogen ion concentration strikingly influences catalase action. Besides this catalase action, ordinary iron

6. Wyckoff: Am. J. Sc. **10**:325, 1925; Ztschr. f. Kryst. **61**:525, 1925. Rinne, F.: ibid. **60**:55, 1924. Heide, F.: Centralbl. f. Min., 1924, p. 641.

7. Welo and Baudisch: Valence Theories and Magnetic Properties of Complex Salts, Nature **116**: 606, 1925.

salts, and particularly certain complex iron salts, possess a strong peroxidase action. In order readily to demonstrate this peroxidase action we employed the benzidine hydrochloride—hydrogen peroxide (blue), the toluidine—hydrogen peroxide (green), the guaiac resin—hydrogen peroxide (dark blue), or the phenolphthalein (pink) color reactions. To detect ferrous iron, we used the ammonium salt of orthonitrosophenol,[8] which gives a beautiful green color with divalent iron even in great dilution. Fresh Saratoga water gives all these color reactions brilliantly. Our attention and interest in mineral water was directed first toward its catalytic activity, and in the experimental part of this article are presented the results of a study of the catalytic activity of the Saratoga springs.

We have considered it necessary to give a thorough theoretical introduction, since we wish to demonstrate to the progressive thinking medical world and to the cultured laity that the whole armor of the modern valence theory and electron theory is required to understand that a new epoch in mineral water therapy is at hand—not only in mineral water therapy, but also in mineral metabolism. It is not, as was formerly believed, the ash analysis which informs us as to the assimilatory value and significance of the physiologic metals, but rather the structure of their salts in the living protoplasm. The structure, however, varies with the environmental air, light, climate, season, geologic nature of the soil, soil bacteria, composition and structure of the spring water, nutrition and other factors. Up to the present, investigations of the physiologic action of inorganic salts have been chiefly concerned with the action of the simple ions, potassium sodium, calcium sulphate, and chlorine, while no attention has been paid to their reciprocal action and to the formation of complex ions. In studying the action of mineral water, we must pay particular attention to this interaction and to the formation of complexes, since we are dealing with a complicated salt mixture. In this connection we can best compare mineral water with sea water to which, as we have already noted, inhabiting plants and animals are most delicately adapted. This adaptation, however, not only runs parallel with the chemical composition of sea water, but also harmonizes completely with the varying physical conditions (pressure, light, oxygen supply and other factors) which are met in the depths. It may be assumed, therefore, that mineral water, too, which is under enormous gas pressure, will have biologic properties different from those of surface water. It is our hypothesis that mineral water suddenly forced to the surface may retain its distinctive physicochemical properties for a short time. This can explain the special activity of water at the spring in contrast to aged water—a fact

8. Baudisch, Oskar and Karzeff, Nikolaus: Ber. d. deutsch. chem. Gesellsch. **45**:1164, 1912.

which has been known for a long time. Methods are still lacking for demonstrating chemically or physically this short-lived condition of a mineral water; yet the researches made by Haertl on water taken at great depths by means of his new deep-sampling apparatus without diminution in gas pressure, give promise of results in this direction. We, therefore, hold to our former idea [9] that the water coming from great depths may possess particular properties which it loses at atmospheric pressure. The foregoing conception does not contradict the recent publication of Fresenius [10] on artificial mineral waters and the direct imitation of natural springs.

Our own experiments with Saratoga water indicate that the ability of a water to maintain its activity in bottles depends to a great extent on the depth from which the water is drawn and on the geologic strata from which it comes. The mere fact that, in a certain sense, mineral

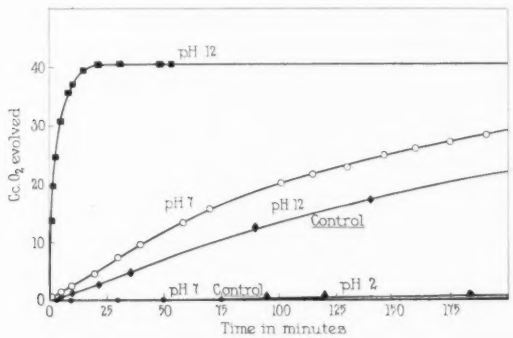

Chart 1.—The results of experiments on the catalase action of sodium aminopentacyano ferroate, $Na_3FeCN_5NH_3$. In each case 10 cc. of O. 356 molar hydrogen peroxide was combined with 0.1 millimol of the iron salt and the evolution of oxygen followed as described by Baudisch and Davidson: J. Biol. Chem. **71**: 501, 1927.

waters possess a structure of which its action is a function, shows that we cannot make any generalizations regarding physicochemical and biologic properties of mineral waters of a different locality. The influence of depth and the significance of radioactive substances in the catalytic and biologic properties of mineral waters forms, however, another study, and will not be dealt with further in this paper.

With regard to the chemical and physical nature of the solid precipitated constituents of mineral waters, previous investigators have indicated only its colloidal nature and have quantitatively related the

9. Baudisch, O., and Welo, L.: Naturwissenschaften, Berlin **13**:755, 1925.

10. Fresenius, L.; Eickler, A., and Lederer, H.: Ztschr. anorg. Chemie **160**: 273, 1927.

size of particle with catalytic activity. We state, on the contrary, that on the basis of the analogies mentioned, the structure of the precipitated submicroscopic crystals must be of the greatest importance. In the calcium carbonate and iron oxide examples we have noted that in one and the same compound it is possible to obtain different lattice arrangements of the atoms in the molecule and, thereby, substances with surfaces of different catalytic activity. We shall go still further and show that even with the same lattice arrangement different substances can be obtained when, as a result of the method of formation, one substance has a perfect lattice while the other lattice structure is not completely developed so that strains in the crystal ensue. In the case of charcoal, Ruff has introduced the concept of active and inactive carbon. The former contains atoms which are not members of the lattice structure, and which bring about the occurrence of so-called residual affinity. If, therefore, we desire to produce crystals with active surfaces, we must direct the empiric preparative methods toward obtaining an incomplete lattice structure or loosening a perfect one.

It is clearly demonstrated, then, that the concept of activity must be connected not only with the size of the crystal, but also with the quality of its development. Our researches on iron oxides have led us to similar results. With the ferromagnetic magnetites, hysteresis measurements are sufficient to demonstrate directly that crystals which are formed quickly and which have no time to grow manifest an internal strain which disappears on heating to red heat. The reactivity of such a strained magnetite, and of the "active" iron oxide derived from it, is considerably greater than that of an unstrained sample in which crystals were formed slowly.

We have discussed the simple iron compounds studied by Baudisch and Welo [11] at some length in order to develop and explain the principal theme of this work, namely, the significance of the structure of simple inorganic compounds in relation to their biologic action. While our previous knowledge of iron and aluminum hydrates has been limited to molecules containing one atom of the metal [$Fe(OH)_3$ and $Al(OH)_3$], the recent investigations of Willstätter [12] show that aluminum forms hydrates in which several atoms of the metal are bound together by oxygen bridges. The selectivity of these structurally different aluminum hydrates is a most baffling phenomenon.

It is interesting that among the iron minerals, several hydrates are found in which the composition is identical: $2Fe_2O_3.3H_2O$ (xanthosiderite, limonite and other minerals), but in which the chemical and physical properties are different. There is a new and fertile field of

11. Welo, L. A., and Baudisch, O.: Philosoph. Mag. **50**:399. 1925; **3**:396. 1927.
12. Willstätter: Ber. **57**:58, 1924.

work for the biochemist in the study of structural differences of inorganic compounds by biologic methods. Such a study would be the basis for the scientific investigation of the therapeutic action of mineral springs. The answer to the question—Can a fresh natural mineral water be artificially reproduced?—is thus self-evident. Only the most exact biologic methods can convince us that our experimental efforts in this direction have succeeded.

In conclusion, we may say that the present state of scientific mineral watertherapy is analogous to that in which the science of fermentation found itself fifty years ago.

Many and varied processes of fermentation became known during the centuries chiefly as a result of empirically established principles which, however, were supported by the observations of outstanding and critical observers. When, however, the cause of fermentation was found to be an organism of one cell and all the knowledge of chemistry, physics, physiology and other sciences began to be utilized, it was possible to put many of the empiric observations on a scientific basis. In this way control was gained over certain biologic processes which previously had to be left to chance.

But here, just as in bacteriology (in which it was originally thought that bacteria alone are sufficient to cause disease), there were disillusionments. While the splitting of sugar was at first ascribed to the metabolism of a single cell organism, the researches of Buchner overthrew this apparently well established fact and made the activity of enzymes known. Recently, however, the great significance of the unimpaired living cell structure in quantitative fermentation has been demonstrated.

The concept that the structure of a reacting substance is of significance is penetrating further and further into biology. In the present work, we have developed the new idea that the structure, and not alone the chemical composition of simple inorganic salts, is important in the course of certain biologic processes.[13]

A systematic study in this direction should be just as fruitful for the problem of mineral springs as the discovery of the cause of fermentation was to that science. The action of mineral water is not merely due to the effect of a complex salt solution, for we know that we must also consider the source of the spring—one might almost say the geology and mineralogy of the spring—in order to understand completely its therapeutic action.

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13. Webster and Baudisch: J. Exper. Med. **41**:473, 1925. Kohlschütter, Von: Die Form des Stoffe im chemischen Vorgang Naturwissenchaften, Berlin **11**:865, 1923.

EXPERIMENTAL WORK

Qualitative Investigations.—For our research the most accessible springs were those of Saratoga in the state of New York. Saratoga possesses numerous springs, but the present investigation has been limited to those known as Geyser, Coesa and Hathorn No. 2. Before describing our experiments, we shall give a brief sketch of the geology of the Saratoga district.

The general geologic condition existing over the whole Saratoga triangle, which takes in a territory about 4 miles square, is as follows: drift, gravel, clay, shale, siliceous limestone, dolomite strata and sandstone. The thicknesses of these various strata are slightly different in the different localities, and at times we find duplications of the strata, due to the upheaval of the west side of the fault.

TABLE 1.—*Chemical Analyses of Mineral Waters at Saratoga Springs, N. Y.**

Chemical Combinations	Hathorn No. 2	Coesa	Geyser
Ammonium chloride	76.49	28.68	45.88
Lithium chloride	75.59	44.27	24.22
Potassium chloride	896.28	506.07	260.77
Sodium chloride	10,569.88	5,558.32	2,387.19
Potassium bromide	72.00	20.00	16.00
Potassium iodide	2.80	2.40	.80
Sodium sulphate	Trace	Trace	Trace
Sodium metaborate	Trace	Trace	Trace
Sodium nitrate	Trace	Trace	Trace
Sodium nitrite	Trace	Trace	Trace
Sodium bicarbonate	1,294.85	614.48	2,047.79
Calcium bicarbonate	3,721.64	2,508.06	1,718.65
Barium bicarbonate	38.88	34.31	16.88
Strontium bicarbonate	Trace	Trace	Trace
Ferrous bicarbonate	14.27	19.62	14.27
Magnesium bicarbonate	2,720.27	1,631.42	851.72
Alumina	23.59	3.19	1.59
Silica	9.80	9.80	13.40
Total	19,516.34	10,980.62	7,399.16

* Made by Reservation Chemists for the State Reservation at Saratoga Springs.

* The results of the analyses are expressed in milligrams per liter. The waters of all the springs are at normal atmospheric temperature and pressure, supersaturated with carbonic acid gas.

At the Coesa spring, we find no drift, gravel or clay; the rock is practically on the surface in this neighborhood, as there are about 9 feet of casing in the seal. The well itself is drilled to a depth of 420 feet. There is a seal in the well at 376 feet, at which point the water is taken in a quantity of about 15 gallons a minute.

The Geyser spring has but little casing in it; the depth of this is unknown, as we have no record giving us these particular data, but the rock at this point is also close to the surface. This surface rock is composed of shale, both in the Geyser district and in the Coesa district. The Geyser spring is bored to a total depth of 144 feet; the seal is at 131 feet, from which point the water is taken in a quantity of about 3 quarts a minute.

All of the waters of Saratoga Springs are found in the dolomite strata. In the case of the Geyser spring, the dolomite is found just under the shale, while in the Coesa spring there are several layers of siliceous limestone superimposed on the dolomite strata. It has been found to be a general experience in Saratoga that wherever most of the bore is located through shale rock, the waters are high in bicarbonate of soda, and that wherever less shale is present and the greater part of the depth of the bore is located in limestone, the waters are more saline and contain more carbonate of lime and magnesia and less bicarbonate of soda.

Catalytic Activity of the Geyser Spring.—This spring is most interesting to us because we had the opportunity to compare the catalytic activity of fresh water from it with that of water that had been stored in white bottles for seven years. In our former paper entitled "On the Aging of Natural Mineral Water," [14] we remarked that aged waters differ from the fresh in a loss of catalytic activity. We have further reported that light promotes aging. These observations were the result of rather crude experiments carried out at the Glauber springs of Franzensbad. These results, however, still stand today, and Heubner,[15] who has carried out analogous experiments on the Pyrmont iron springs, arrived at similar results. Since the earliest investigations of the destructive action of mineral waters on hydrogen peroxide the statement has repeatedly appeared that this property is noted only in fresh waters. We were, therefore, much astonished to find that Geyser water which had been stored for seven years in colorless bottles without any particular protection from the light still showed a strong peroxidase or catalase activity. It is noteworthy that in the course of years no precipitate formed in these bottles as is usually the case in old mineral water. The clear contents gave all the previously mentioned peroxidase color reactions intensely and showed a brilliant green color with orthonitrosophenol, which indicated the presence of ferrous iron. It was striking that the water taken directly from the spring did not react so intensely as the water which had aged several years. Acidified potassium ferricyanide failed to indicate the presence of ferrous iron. In the absence of air the solution remained unchanged, and only in an open tube did the color change on standing from yellow to light blue. An artificial ferrous bicarbonate solution of like iron content (0.0260 Gm. per liter) gave an immediate pale blue with potassium ferricyanide. If Geyser water is irradiated for a short time in the presence of traces of oxygen, it gives an immediate blue with acidified ferricyanide. While orthonitrosophenol is capable of indicating the ferrous iron of the catalytically

14. Baudisch, O., and Welo, L. A.: J. Biol. Chem. **64**:753, 1925.

15. Heubner: Ztschr. f. Wissenschtl. Bäderkunde **1**:74, 1926.

active Geyser water in that it forms a green colored complex ferrous orthonitrosophenol, no Prussian blue is formed by ferricyanide in the absence of oxygen. By means of these color reactions, it was primarily established that an aged mineral water does not necessarily lose its peroxidase property; the opposite has previously been taken to be the fact.

There was a high pressure of carbon dioxide in the old Geyser water bottles, and it might have been assumed that the catalytic power was related to the dissolved carbon dioxide. Mineral water was therefore placed in a suitable separatory funnel with a neck 20 cm. long and 3 cm. wide, air being excluded. The water was covered with white petrolatum to a height of several centimeters. The excess of carbon dioxide escaped through the petrolatum which was maintained in liquid state, and completely shut out oxygen. In twelve hours the bubbling of gas ceased. The water in the funnel was still clear and gave all the peroxidase color reactions just as intensely as the original carbon dioxide containing water. The contents of the funnel were then heated nearly to boiling. Under these conditions, the water became cloudy due to the decomposition of bicarbonates. The funnel was allowed to stand for five days, during which time a gradual precipitation of solid constituents began. The surface remained continuously covered with petrolatum, however, while the stopcock of the funnel was kept under alkaline pyrogallol solution. When tests were then made with benzidine hydrochloride —hydrogen peroxide, the color reaction was blue; with phenolphthalin, deep red; with guaiac resin, deep blue and with orthonitrosophenol, brilliant green.

This experiment brought the fact that neither the dissolved nor the bicarbonate carbon dioxide was necessary for the color reactions.

EFFECT OF LIGHT ON MINERAL WATER AND FERROUS BICARBONATE SOLUTIONS

Three parallel light experiments were now undertaken, which immediately gave the clue to the explanation of the loss of catalytic reactions previously observed. Three quartz test tubes which had been drawn out to capillaries at the upper end were employed. Two of these were filled about three-fourths full (*A* and *A'*), while the third (*B*) was completely filled in the absence of air so that the liquid reached to the capillary. The three tubes were then sealed off in the oxyhydrogen blowpipe. *A* and *A'* had not been rinsed with carbon dioxide as had *B*. *A* and *B* were then irradiated with a carbon arc light for nearly two hours while being cooled with running water at 13 C. At the end of this time the contents of the tubes were slightly opalescent. Tests of the two tubes gave entirely different results. In *A* all the peroxidase reactions had disappeared, and orthonitrosophenol gave a reddish color

instead of the green, indicating that ferric iron was present. The opalescent contents of *B* gave all the peroxidase reactions intensely as well as a green color with orthonitrosophenol. The contents of *A'*, which had been kept in a dark incubator during this time, were only slightly opalescent, while all the color reactions were considerably weaker than originally. *A* and *A'*, which contained traces of oxygen, differed extraordinarily after the irradiation, although more of the excess of carbon dioxide could escape and considerable pressure of carbon dioxide was present in the tubes. Light, then, effected the complete oxidation of the ferrous iron in the tube which contained oxygen, whereupon all the peroxidase reactions disappeared.

Although *A'* had been subjected to a higher temperature than its irradiated duplicate, it retained its catalytic properties to some extent. The oxidizing power of oxygen is enhanced by light energy, as can better be seen from the quantitative experiments to be described. In *B*, in which no trace of oxygen was present, the catalytic properties were completely retained, although a visible opalescence occurred. This signifies an increase in the size of the particles which originally were not even visible by the Tyndall phenomenon.

These qualitative experiments established the fact that the short wave lengths of the light of the carbon arc, to which quartz is transparent, exert an influence on Geyser water in the absence as well as in the presence of oxygen. In the absence of oxygen no oxidation of the ferrous irons occurs, and, therefore, no decomposition of water. As further evidence of this fact, a solution of ferrous pyrocatechol was prepared. In the absence of oxygen this solution is colorless. Irradiation for one hour with the carbon arc did not cause the least apparent change. If, however, a small amount of potassium nitrate were added and the solution again irradiated, an intense violet color occurred in a few minutes. Ferric pyrocatechol, which is similar to permanganate in color, was formed by the oxygen split off from the nitrate.

We have already indicated in the described experiments with potassium ferricyanide that the ferrous iron in Geyser water does not always behave like that in ferrous bicarbonate. It was therefore of interest to study the behavior of a solution of ferrous bicarbonate on irradiation. For this purpose a ferrous bicarbonate solution containing 0.178 Gm. of ferrous iron per liter was prepared. When this solution was irradiated while being cooled in running water, it soon developed a yellowish clouding, and finally a precipitate settled out which was reddish brown and consisted of ferric carbonate. After an hour's irradiation, the water still contained 0.05584 Gm. of ferrous iron per liter.

This experiment indicates that ordinary ferrous bicarbonate solutions are sensitive to light, decomposing water with the formation of

hydrogen, while the ferrous salt is oxidized to ferric carbonate. In this case, therefore, the iron ion in ordinary ferrous bicarbonate behaves differently from the complex ferrous ion in mineral water. A solution of ferrous bicarbonate prepared from the purest iron (from iron carbonyl), water and carbon dioxide, containing 25 mg. of iron per liter (as does the Geyser water) gives the reactions with benzidine, toluidine, guaiac resin and orthonitrosophenol just as strongly as does Geyser water, but it gives no reaction with phenolphthalin, though all the catalytically active springs which we have investigated give, in the presence of air (but without hydrogen peroxide), a precipitate of zinc carbonate which is at first rose-colored and which quickly becomes intense red. If, however, the divalent iron in mineral water is transformed into the ferric form by means of traces of air and intense irradiation, then only the flocculent white precipitate of zinc carbonate and no red color, occurs. This indicates that the red coloration depends on the presence of active divalent iron and not on the other inorganic salts. It is this element alone which is capable of quickly absorbing and activating atmospheric oxygen so that the colorless phenolphthalin becomes oxidized to phenolphthalein which, in the alkaline solution, forms its familiar red potassium salt. Neither dilute nor concentrated ferrous bicarbonate solutions give a red color with phenolphthalin (with or without hydrogen peroxide). In the absence of peroxide, the concentrated solution gives a green precipitate of ferrous hydroxide while in the presence of hydrogen peroxide all the iron is instantly converted to the yellowish-red ferric hydroxide without the phenolphthalin.

Phenolphthalein mixed with H_2O_2 forms the well known blood reagent of Kastle and Meyer,[16] which is sensitive to blood as dilute as 1:800,000. This reagent is of particular interest in connection with the catalytic properties of mineral waters, since in them the red coloration occurs in the absence of H_2O_2 and also because oxygen or air is necessary besides divalent iron. As long as the mineral waters investigated gave a green color with orthonitrosophenol (Fe^{II}) a red color was obtainable with phenolphthalin, whether the water contained an excess of carbon dioxide or was heated nearly to boiling.

Quantitative Investigations of Geyser Spring.—The method of investigation of the catalase action of mineral water was developed by Glénard.[17] The following is the method as employed by us:

16. Glaister, John: Brit. M. J. **1**:650, 1926. Preparation; phenolphthalein (2 Gm.), potassium hydroxide (20 Gm), and water to make 100 cc. are boiled while zinc dust (from 10 to 30 Gm.) is added until the solution is decolorized after boiling ten minutes. The solution keeps well if a little zinc is present.

17. Glénard: Proprietes physicochimiques des eaux de Vichy, Paris, 1911.

Two hundred cubic centimeters of mineral water or the solution to be investigated was quickly brought to 37 C. by immersion in a hot water bath and mixed with 25 cc. of approximately half molar peroxide (at 37 C.). The peroxide was freshly prepared by diluting Merck's perhydrol. The time of mixing was noted and the solution then maintained at 37 C in the incubator. After fifteen minutes, 25 cc. of the mixture was pipetted out, acidified with sulphuric acid and titrated with tenth normal potassium permanganate. The titration was repeated at definite intervals. The initial hydrogen peroxide (H_2O_2) concentration in the mixture was determined by means of a control with distilled water. Table 2 and charts 2 and 3 which show the extent and rate of peroxide decomposition by Geyser water yield quantitative confirmation of the qualitative color reactions. In our case, peroxidase and catalase reactions seem to run parallel.

TABLE 2.—*Catalytic Properties of the Geyser Spring Water*

	Time (Minutes) After the Beginning of the Experiment	Cc. Tenth Normal Potassium Permanganate	a–x	$\frac{a}{a-x}$	$\frac{1}{t} \log \frac{a}{a-x}$
(I)	0	29.35	....	.....	.....
	15	.....	19.9	1.473	0.0113
	45	.....	7.9	2.520	0.0134
	90	.....	4.6	1.717	0.0047
	121	.....	3.2	1.437	0.0051
(II)	0	30.65	....	.....	.....
	17	.....	24.70	1.242	0.0056
	32	.....	19.52	1.264	0.0068
	62	.....	10.88	1.795	0.0085
	91	.....	6.75	1.610	0.0071
	121	.....	4.80	1.406	0.0049
(III)	0	29.13	....	.....	.....
	14	.....	22.7	1.285	0.0078
	31	.....	15.7	1.447	0.0094
	60	.....	6.9	2.28	0.0123
	91	.....	4.2	1.64	0.0069
	121	.....	3.2	1.31	0.0039
(IV)	0	32.5	....	.....	.....
	15	.....	30.9	1.051	0.0014
	30	.....	28.5	1.087	0.0024
	45	.....	24.7	1.153	0.0041
	75	.....	16.6	1.481	0.0057
	105	.....	8.8	1.886	0.0092
	165	.....	4.6	1.913	0.0047

A comparison of our results with the data on European springs brings out a striking difference in both the extent and the velocity of peroxide decomposition. The Geyser spring and, as we shall show later, the other springs, are extraordinarily active. Our observation that water stored in bottles for seven years retains all of its activity is novel; yet it is easily explained in view of other results of our investigation. Geyser water is drawn from pipes over 100 feet deep and is bottled in the complete absence of air, the bottles being preliminarily subjected to careful rinsing with air-free carbon dioxide. The secret of the preservability of the water lies in the ingenious machine which maintains it free from oxygen until it is bottled. The smallest trace of oxygen would, during long storage, gradually oxidize the complex ferrous iron

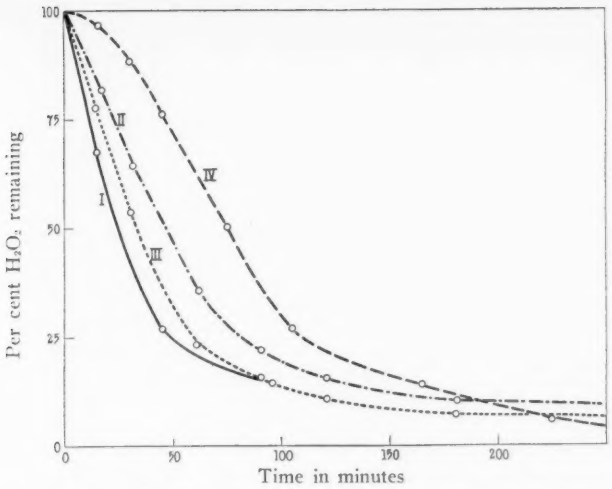

Chart 2.—The catalytic properties of the Geyser Spring (fresh and 7 year old water). Curve I indicates Geyser water from bottles filled on Jan. 3, 1920, and heated to 85 c. in the absence of air; curve II, Geyser water from bottles filled Jan. 3, 1920, with the carbon dioxide shaken out before measurement; curve III, Geyser water from bottles filled on Jan. 3, 1920; and Curve IV, Geyser water measured at the spring on Jan. 2, 1927.

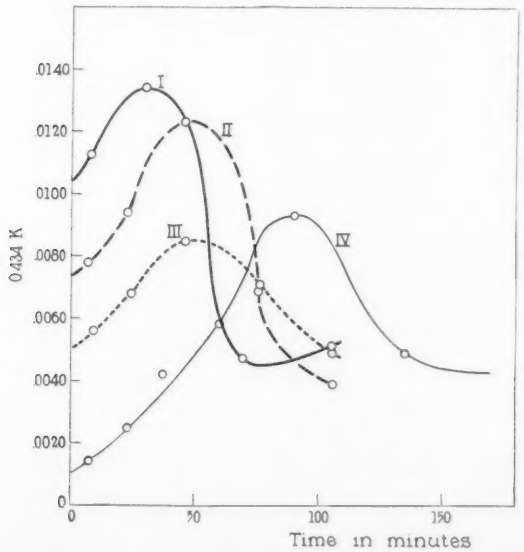

Chart 3.—The catalytic properties of the Geyser Spring (fresh and 7 year old water). Curve I indicates Geyser water from bottles filled on Jan. 3, 1920 and heated to 85 c. in the absence of air; curve II, Geyser water from bottles filled on Jan. 3, 1920, with the carbon dioxide shaken out before measurement; curve III, Geyser water from bottles filled on Jan. 3, 1920; curve IV, Geyser water measured at the spring on Jan. 2, 1927.

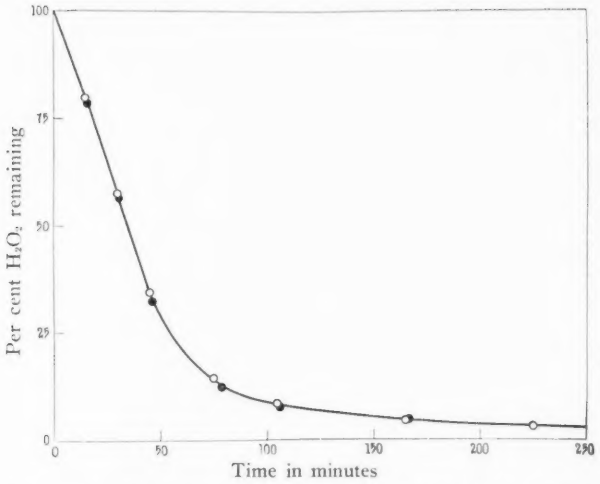

Chart 4.—The catalytic properties of Hathorn Spring No. 2. The white dot indicates results of water measured at the source on Feb. 3, 1927; the black dot indicates results of water bottled Dec. 7, 1926.

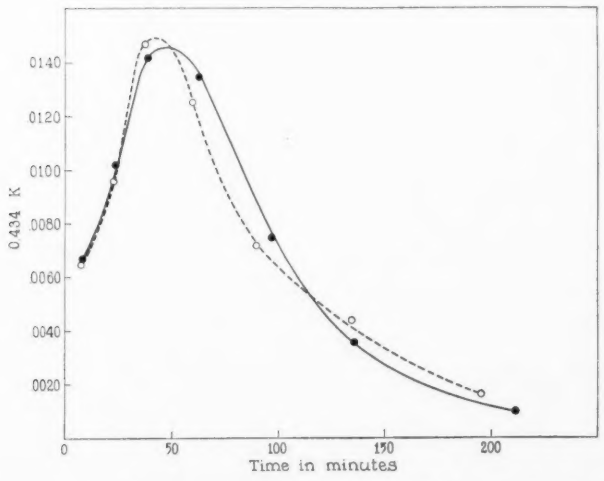

Chart 5.—The catalytic properties of the Hathorn Spring No. 2. The broken line indicates results of water measured at the source Feb. 3, 1927; the unbroken line indicates results of water bottled Dec. 7, 1926.

to ferric, thereby causing precipitation and loss of catalytic properties. Bottles which show the presence of a precipitate have weak peroxidase-catalase properties or none at all.

It is also seen from our experiments that the catalyst can be precipitated without losing its activity if oxygen is absent. This gives us a splendid demonstration of the fact mentioned previously, namely, that it is not the size of particle but the structure of the solid substance which is important for its catalytic activity. Up to the present it has been assumed that the catalytically active substance in mineral water is molecularly or at least colloidally dispersed and as a result of this dispersion shows an unspecific surface action.

The Catalytic Activity of Hathorn No. 2 Spring.—In charts 4 and 5 is given a representation of the catalytic decomposition of H_2O_2 by

TABLE 3.—*The Catalytic Properties of Hathorn No. 2 Water*

	Time (Minutes) After the Beginning of the Experiment	Cc. Tenth Normal Potassium Permanganate	a–x	$\frac{a}{a-x}$	$\frac{1}{t} \log \frac{a}{a-x}$
(I)	0	32.5	....	.....	.....
	15	.....	25.9	1.254	0.0065
	30	.....	18.6	1.392	0.0096
	45	.....	11.2	1.660	0.0147
	75	.....	4.7	2.380	0.0125
	105	.....	2.8	1.678	0.0072
	165	.....	1.5	1.866	0.0044
	225	.....	1.2	1.250	0.0016
(II)	0	30.16	....	.....	.....
	16	.....	23.6	1.279	0.0067
	30	.....	17.0	1.389	0.0102
	47	.....	9.75	1.743	0.0142
	78	.....	3.72	2.620	0.0135
	106	.....	2.3	1.618	0.075
	166	.....	1.4	1.643	0.0036
	258	.....	1.14	1.228	0.0010

Hathorn water. It is noteworthy that water three months old had not suffered any loss in catalytic activity. As may be seen from its chemical analysis, Hathorn No. 2 is a highly mineralized water, and in this respect is different from the Geyser spring.

Influence of Light on the Coesa Spring.—In connection with the qualitative reactions of the Geyser spring, we have already explained why the carbon arc light sometimes causes an increase or at any rate does not cause a decrease in the catalytic activity of the water, while in other cases the activity is completely lost.

In the study of the action of light on mineral springs, little attention has previously been paid to traces of oxygen. Fresenius [10] says that precipitates are observed in irradiated solutions only when they contain air. He did not observe an influence of light on the catalysts. Schoeller and Rothe [18] do not believe that there is a relation between the

18. Schoeller and Rothe: Klin. Wchnschr. **6**:348, 1927.

influence of light and the disappearance of catalytic properties of natural mineral water. They assume, further, that the state of the ferrous bicarbonate or rather the presence of the system, $Fe^{II} + HCO_3^- + CO_2$, is responsible for the benzidine reaction and the continuance of the activity. According to their idea, the partial pressure of CO_2 and a lack of oxygen are important for the maintenance of the catalytic activity of a natural curative water. How little all their assumptions harmonize with our experimental results is apparent. That the presence of air diminishes and finally completely destroys the activity of a natural mineral water is a phenomenon which has been known since ancient times.

We have used the carbon arc in our researches because its spectrum approaches most closely that of the sun, and the longer ultraviolet wave

TABLE 4.—*The Catalytic Properties of Coesa Water*

	Time (Minutes) After the Beginning of the Experiment	Cc. Tenth Normal Potassium Permanganate	a−x	$\frac{a}{a-x}$	$\frac{1}{t}\log\frac{a}{a-x}$	P_H
(III)	0	31.2	....	.....	.....	..
	20	....	29.5	1.058	0.0012	7
	45	....	25.7	1.149	0.0024	..
	75	....	20.1	1.279	0.0036	..
	115	....	12.6	1.594	0.0023	..
	165	....	5.2	2.422	0.0077	..
(IV)	0	31.2	....	.....	.....	7
	20	....	30.8	1.013	0.0003	..
	45	....	29.8	1.033	0.0006	..
	75	....	28.6	1.042	0.0006	.
	115	....	26.6	1.074	0.0003	..
	165	....	22.8	1.122	0.0010	..

lengths are present in abundance. Preliminary tests showed that the ultraviolet portion of the sun's spectrum was the most effective in our experiments. Later we shall analyze the most active wave lengths in detail.

For our experiments we used Coesa water which had been bottled on Dec. 12, 1926. We had, therefore, a uniform supply of water and could carry out our experiments with all convenience in the laboratories of the Rockefeller Institute, instead of going to the source of the spring. The water gave all the peroxidase color reactions intensely.

Apparatus: The previously described long-necked separatory funnel with a capacity of about 1.5 liter was used. One of these was made of fused quartz and was used for the irradiation experiments. The following parallel experiments, which are best expressed in the charts, were carried out.

Experiments in the Presence of Traces of Oxygen: The contents of four bottles of Coesa water (about 2 liters) were combined in a large beaker from which the water was poured into two separatory funnels, one of glass (*A*) and

one of quartz (*B*). During this operation the mineral water came in contact with air. The water in the funnels was then covered with petrolatum to a height of 4 inches (10.16 cm.), which sealed it from the air. The funnels were cooled with running water (13 C.), *A* remaining in diffused daylight while *B* was irradiated for one hour with the carbon arc light. Both funnels were then placed in a dark incubator (37 C.) and allowed to remain for three hours. At the end of this time, the water in *A* was slightly opalescent while that in *B* was cloudy. The p_H was 7 in both vessels. The results of the measurements of the catalase action are given in table 4.

Experiments in the Absence of Oxygen: A glass (*C*) and a quartz (*D*) funnel were each filled with the contents of two bottles in the complete absence of air. For these experiments the funnels were strongly heated with Bunsen burners to remove occluded gases, while carbon dioxide, carefully freed from

TABLE 5.—*Subsequent Determination of the Catalytic Properties of Coesa Water*

	Time (Minutes) After the Beginning of the Experiment	Cc. Tenth Normal Potassium Permanganate	a-x	$\frac{a}{a-x}$	$\frac{1}{t}\log\frac{a}{a-x}$	p_H
(I)	0	27.8	....	.....	.....	..
	15	....	24.3	1.142	0.0038	7
	25	....	21.5	1.130	0.0053	..
	45	....	14.7	1.463	0.0083	..
	75	....	6.8	2.162	0.0112	..
	110	....	2.6	2.613	0.0119	..
(II)	0	27.8	....	.....	.....	..
	15	....	25.8	1.077	0.0021	7
	25	....	23.9	1.080	0.0033	..
	45	....	19.0	1.258	0.0005	..
	75	....	10.1	1.881	0.0091	..
	110	....	3.5	2.885	0.0131	..
(V)	0	26.8	....	.....	.....	..
	15	....	23.4	1.144	0.0039	..
	30	....	18.9	1.239	0.0062	..
	60	....	10.8	1.750	0.0081	..
	100	....	5.0	2.160	0.0084	..
	115	....	3.9	1.281	0.0071	..

oxygen, was passed through them for an hour. The funnels were filled while still warm, the vigorous effervescence of the mineral waters hindering the access of air. They were then sealed with melted white petrolatum, through which the excess CO_2 easily passed. The stopcocks of the funnels were kept under the surface of alkaline pyrogallol solution. After forty-eight hours at room temperature, the evolution of carbon dioxide had ceased, both funnels remaining clear. *D* was then irradiated for one hour, while being cooled with running water (13 C.), when it became distinctly opalescent. Both funnels were placed in the incubator for four hours to attain the necessary temperature. The p_H in both was 7. The results of the subsequent determination of catalytic properties are given in table 5.

The experiments in the presence of oxygen only confirm what was previously found by means of peroxidase color reactions in the case of Geyser water. Light considerably accelerated the oxidation of ferrous ion. Hydrogen ion concentration plays no part in this process because

it, as well as all other conditions, was identical in both cases. Qualitative tests of the water in the two funnels confirmed the result mentioned before. In the unilluminated funnel all the previously mentioned color reactions were positive but weak. The illuminated funnel, on the contrary, did not show any of these reactions, and orthonitrosophenol indicated ferric iron.

The fact that light accelerates the oxidation of ferrous ion by molecular oxygen without at the same time photochemically decomposing water, is of biologic interest. The assumption that in the mentioned

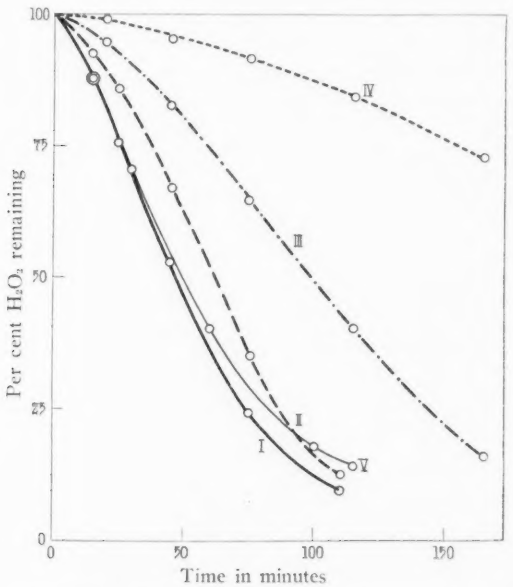

Chart 6.—The influence of light on the catalytic properties of Coesa Water (bottled Dec. 1926) in the absence and in the presence of oxygen. Curve I indicates oxygen-free water, irradiated; curve II, oxygen-free water, not irradiated; curve III, water with traces of oxygen not irradiated; curve IV, water with traces of oxygen, irradiated, and curve V, water direct from the bottle.

experiments light does not form hydrogen peroxide from water arises from the results of the oxygen-free illuminated experiment (*D*). If hydrogen peroxide were formed from water by irradiation of mineral water, the ferrous complex would become oxidized, as is the case with ferrous bicarbonate.

The oxygen-free experiment, however, leads to another question which is of fundamental significance for the action of light, namely, the "significance of light for the stability of colloidal solutions." A

large amount of literature already exists on this subject, and this is not the place to discuss it more thoroughly. We are interested only so far as our experimental results are connected with the theme already discussed in the theoretical part of this article, namely, the size and structure of the particle. Up to the present time, the catalytic action of a mineral water has always been related to and explained by the so-called molecularly dispersed or generally colloidal state of the iron or manganese.[19] Our experiments with light in oxygen-free water show that light brings about a change in dispersion which finally leads to the pre-

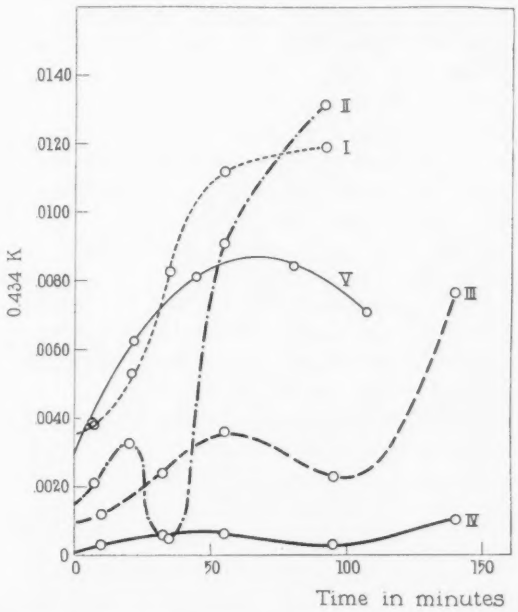

Chart 7.—The influence of light on the catalytic properties of Coesa Water (bottled December, 1926) in the absence and in the presence of oxygen. Curve I indicates oxygen-free water, irradiated; curve II, oxygen-free water, not irradiated; curve III, water with traces of oxygen, not irradiated; curve IV, water with traces of oxygen, irradiated, and curve V, water direct from the bottle.

cipitation of the disperse phase (coagulation). Thus, particles which were originally invisible increased in size and became visible under the influence of light. Water was not photochemically decomposed in the process, since such a change would have led to the oxidation of ferrous ion. If the size of the particle alone were responsible for the catalytic activity, the clear unilluminated water should have been more active than the illuminated, which is contrary to the facts. The precipitated particles

of ferrous compound must possess a surface with specially active points, since the reaction velocity of hydrogen peroxide decomposition is greater than that with the original molecularly dispersed particles. We see, therefore, that it is justifiable, in the mineral water problem, too, to enlarge on the idea that the process of formation of an inorganic salt is a function of its catalytic surface action.

Even if, on the basis of previous experimental results, we are not able at present, to say definitely that the divalent iron in the Saratoga springs is bound in a structurally defined and isolatable complex salt, it is to be seen that a specific character is contributed to the ferrous ions by their companions in solution, a character which is not dependent on contained gases, particularly carbon dioxide, and which differentiates it from the ferrous ion in ferrous bicarbonate. While ferrous bicarbonate is rapidly oxidized to ferric carbonate by the oxygen resulting from the photochemical decomposition of water, mineral water appears to use the light only for diminishing the charge on its colloidal particles and thereby increasing their mass. To what extent this process can be explained by the change in the dissociation of electrolytes contained in mineral water must be decided by further experiments. It may be said, however, that light influences the dispersion of the colloidal particles in mineral water and thereby its catalytic activity. In the light of modern colloid chemistry, colloidal particles are no longer to be considered simple particles which show unspecific absorption phenomena because of their large surface, but, rather, structurally defined crystals in which the previously mentioned laws of valence find just as important application as in the electrolytes and in the complex salts.

SUMMARY

1. The Saratoga springs investigated by us displayed a much higher catalytic activity than any of the European springs previously investigated.

2. It was observed that bottled waters retain their catalytic activity if every trace of oxygen is excluded in the bottling.

3. The catalytic activity of the Saratoga waters depends primarily on their ferrous iron content, which is most probably in complex form. It is independent of dissolved or bicarbonate carbon dioxide.

4. The mineral waters lose their catalytic activity in the presence of oxygen, especially when irradiated by the carbon arc light.

5. In the absence of oxygen, heat and light alter only the dispersion of the contained colloid particles without weakening the catalytic power.

We wish here to express our thanks to Dr. Herbert Ant, State Chemist at Saratoga Springs, to whom we are indebted for determinations of the catalytic action of the mineral waters at the source, as well as for many helpful suggestions.

MOTION OF THE HEART IN DISEASE OF THE MITRAL VALVE

CINEMATOGRAPHIC ROENTGEN-RAY STUDIES *

W. EDWARD CHAMBERLAIN, M.D.
AND
WILLIAM DOCK, M.D.
SAN FRANCISCO

Roentgenograms of the heart which do not record the rhythmic movements of its borders must always remain of limited value. Even the orthodiagram, while offering information not to be obtained from the roentgenograms, fails to inform us of the time relations of the movements of various parts of the heart outline.

In 1925, Ruggles [1] succeeded in making 8 by 10 inch instantaneous roentgenograms of the beating heart, serially, at the rate of fifteen per second. He reduced these roentgenograms, photographically, on to a motion picture film. When projected, Ruggles' film gave an excellent demonstration of the shadow of the moving heart, with detail and visibility far exceeding the best that can be obtained by watching the shadows on the fluorescent screen.

Any one who has observed the movements of the heart, as shown by the fluoroscope, or with the greater clarity of Dr. Ruggles' cinematographic demonstration, must appreciate how valuable would be a graphic record of the in and out movements of selected parts of the border of the heart. While watching the demonstration of Ruggles' motion picture, it occurred to us that such a graphic record could be obtained by measuring corresponding dimensions on successive films in any series taken with Ruggles' roentgen cinematograph.

The resulting studies and curves of heart border motion (Skiacardiograms) obtained from Ruggles' original films of a normal subject, have been reported elsewhere.[2] We have now produced similar records in cases of valvular disease.

In applying this method of analysis of the movements of the heart border to the diseased heart, a number of difficulties were at once encountered. The present apparatus of Ruggles for obtaining the necessary rapid-sequence roentgenograms does not give satisfactory

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1. Ruggles, H. E.: X-ray Motion Pictures of the Thorax, Radiology. **5**:444 (Nov.) 1925.

2. Chamberlain, W. E., and Dock, W.: The Study of the Heart Action with the Roentgen Cinematograph, Radiology. **7**:185 (Sept.) 1925.

films of thick-chested patients, and it cannot be used with nervous or bedridden patients. By using high voltage, the value of which has been demonstrated by Lomon and Commandon,[3] and by enclosing the film-carrying mechanism in a separate soundproof compartment, the application of this method could be widely extended.

We have used the original machine on four patients with disease of the mitral valve; this disease was considered especially important because the mechanics of the valve lesion are not easily elucidated by the ordinary methods of study, and it seemed that such cases might give a fair idea of the value of graphic records of heart movement.

TECHNIC

The film-carrying apparatus was loaned to us by Dr. Ruggles of the University of California, and operated with the assistance of Mr. E. P. Fletcher. The method of operating this machine and of measuring the films and plotting graphically the motion perpendicular to the border of the heart of various points in the auricular and ventricular areas has been described elsewhere.[2] Thirty feet of duplitized film are taken through the film carrier in two seconds, giving thirty exposures of one-fortieth of a second's duration each. However, due to the technical difficulties in runing 30 feet of film through the present machine without mishap, ten rolls (300 feet) of film were used on these four cases. We have twenty-one consecutive exposures on case 1, twenty-four and twenty-six consecutive exposures on case 2, twelve and nineteen on case 3 and ten consecutive exposures on case 4. This gave us 3 heart cycles of case 1, a total of 5 cycles of case 2, a total of 2.8 cycles of case 3 and 1.3 cycles of case 4. The roentgenograms of cases 2 and 4 were satisfactory for accurate measurement. In case 1 the heart was so large that the right border is not on the films (8 by 10 inches [20 by 25 cm.]). In case 3 the hilum and lung density interfered seriously with accurate measurement, so that the error was twice as great as in the normal or in the other three cases. The curves obtained from the two sets of film of this case were similar, and we felt that a good approximation of the motion of the heart had been reached.

REPORT OF CASES

Case 1.—Mr. G. H., aged 35, had had severe rheumatic fever in childhood, with mild recurrences almost annually during youth. He had been in a hospital for six months at the age of 24 for rheumatic fever, and for eight months at the age of 25 because of coughing and dyspnea. From the age of 26 to 31 he had worked hard (truck-driver), but had been in a hospital again for four months for cough and dyspnea. He returned to his previous work and continued at it until he entered the clinic, although for the last two years of that time he had had constantly recurring dyspnea and palpitation on exertion, and, at intervals of a few weeks, had had aching pain in the epigastrium for from two to three days at a time. This pain was worse two hours after meals and was relieved by rest. It became severe, and he entered the clinic eight months before our observations were made. The liver was tender and extended 4 cm. below the right costal margin. There were no râles at the bases of the lungs. He was in a hospital for one month and returned to light work. Three months later he had acute bronchitis, with fever for ten days, and was in Lane Hospital for two weeks. He then returned to work, but had rather marked palpitation even on moderate exertion.

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3. Lomon and Commandon: La cinematographie radiologique, J. de radiol. et d'electro. **8**:433 (Oct.) 1924.

Physical Examination.—The patient was a thin but muscular man. There was a definite malar flush and slight cyanosis of the lips. The apex beat in the left anterior axillary line was forceful, rapid and irregular, due to frequent extrasystoles; there was no thrill. The apical first sound was replaced by a harsh, loud systolic decrescendo murmur. The second sound was faint at the apex but loud at the pulmonic area. There was a soft low-pitched diastolic murmur, heard only outside the apex over a small area. It was loudest early and late in diastole, but audible throughout the entire diastole. The lungs were clear, the liver was not palpable. The diagnosis was rheumatic heart disease and mitral stenosis.

The electrocardiogram showed right ventricular preponderance. The vital capacity was 55 per cent. (Autopsy, eight months after roentgen cinematography showed that the mitral orifice consisted of a scarred and calcified opening of decidedly fixed size with a circumference of 7 cm. Regurgitation through such an orifice must have been marked.)

Case 2.—Mrs. T. G., aged 19, had not had rheumatic fever or chorea, but she had had severe "growing pains" for years during childhood. She lived on one of the highest hills in San Francisco and experienced slight dyspnea on walking up to her home. She had been married one year and had not been pregnant. For three months she had had severe palpitation on exertion or during excitement.

Physical Examination.—The patient was small, fairly well nourished and of light frame. The coloring of the face was a little high. The tonsils were markedly enlarged, reddened and cryptic. (They were removed under local anesthesia, without any untoward sequelae, three weeks after our observations.)

The apex beat was in the fifth left interspace, just outside the midclavicular line. It was sharp, forceful, regular and 90 a minute. At the apex the first sound was loud and snapping, the second sound was fairly loud, and there was a high pitched, intense systolic murmur, sharply distinguished from the heart sounds but filling all of the systole. It was heard well in the axilla and was not transmitted to the midline, although audible along the left sternal border and in the pulmonic area. With the patient in a recumbent position, a soft, low presystolic murmur was heard in the fourth interspace, about 2 cm. inside the apex. The pulmonic second sound was sharply accented. The diagnosis was mitral stenosis.

The electrocardiogram was normal; the vital capacity was 72 per cent.

Case 3.—Mr. A. H., aged 28, had had rheumatic fever at the age of 5 years and again at the age of 12. There had been some dyspnea and palpitation on exertion since childhood. From the age of 16 to the age of 24 he did hard manual work (mill hand). For the next four years, he did only light work. For six months, he was increasingly short of breath and had several attacks of nocturnal dyspnea. He felt weak, and was unable to work; he then had acute bronchitis with fever for a week. He entered Lane Hospital at that time, remained one week after his fever subsided, and was then in a convalescent home for three weeks. He felt weaker than before his acute infection, and had had two attacks of nocturnal dyspnea during the week preceding our observation.

Physical Examination.—The patient was a rather small, thin man, with definite cyanosis of the lips and fingertips. The apex beat was scarcely palpable, but there was a long purring diastolic thrill in the fourth and fifth left interspaces from 7 to 1.2 cm. from the midsternal line. The first sound at the apex was sharp and fairly loud. There was only a soft systolic murmur at the apex, not well heard over the rest of the precordium. There was a loud continuous rumble filling all of the diastole. The pulmonic second sound was slightly accentuated. There were fine râles at the bases of both lungs. The diagnosis was rheumatic heart disease, and mitral stenosis.

The electrocardiogram showed right ventricular preponderance, abnormally broad and high P waves. The vital capacity was 60 per cent.

CASE 4.—Mr. C., aged 28 (private patient of Dr. A. W. Hewlett and Dr. J. K. Lewis), had had rheumatic fever at the age of 19; valvular disease of the heart was demonstrated the following year. Dyspnea on exertion increased gradually. At the age of 25 it became marked, and he had had hemoptysis on three occasions. One year later, auricular fibrillation commenced, and regular rhythm was twice restored with quinidine. After fibrillation had occurred for the third time, Dr. Hewlett discontinued the use of quinidine and prescribed digitalis. A right hemiplegia with aphasia came on during the night one month later. It gradually cleared up in about a week, but occasional slight dragging of the right foot remained. The patient was able to continue with his studies, though he tired easily and had occasional nocturnal dyspnea, as well as dyspnea on exertion.

Physical Examination.—The patient was thin, with a slight frame and muscles. There was moderate cyanosis of the lips, ear lobes and fingers. The apex beat was in the anterior axillary line, absolutely irregular, about 95 a minute. There was a faint diastolic thrill and a continuous diastolic rumble, together with a soft systolic murmur, at the apex. The pulmonic second sound was loud, and there was a faint early diastolic murmur to the left of the sternum in the third interspace. The diagnosis was rheumatic heart disease, mitral stenosis, aortic insufficiency and auricular fibrillation.

The electrocardiogram showed auricular fibrillation and a normal ventricular complex.

CURVES ON ROENTGENOGRAMS OF THE HEART

The curves obtained by plotting the motion of points in the cardiac border against the time (one-fifteenth second for each exposure) are compared with the normal curves obtained from the roentgenograms of Dr. Ruggles' subject. Noteworthy variation from the normal is not found in the ventricular areas. In the normal the upper part of the left ventricle continues to move mesad in early diastole, while the apex moves rapidly laterad. This is observed only in case 2, and there only for the first one-fifteenth of a second. We anticipated finding a change in the position of the "shoulder" on the diastolic rise in ventricular distention, but notable change is not found. Again we must emphasize the fact that the curve of motion of points on the ventricle does not in any way resemble the curve of volume changes in the ventricle as determined by the plethysmograph in animals, and that great variation is seen in motion of different points along the left ventricle, so that comparison of one case with another may be misleading. The total extent of motion of the left ventricle in cases 1 and 2 (chart 1) was roughly the same as in the normal; in case 3 (chart 2) and case 4 it was diminished. The right border moved in phase with the apex in cases 2, 3 and 4, though the total extent of motion in case 3 was slight (1.5 mm.).

In case 4 we can find no point in the auricular area which moved out of phase with the ventricle. Dr. J. K. Lewis points out to us that this is probably due to thrombosis in the left auricle. It will be recalled that this patient had had cerebral embolism two years previously.

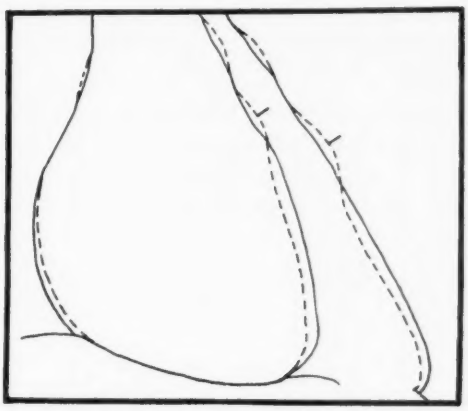

Chart 1.—The curves on the right indicate the left border of the heart in case 1; on the left is shown the cardiac outline in case 2. The straight line denotes the end of diastole; the broken line, the end of systole. Note the prominence of the left auricular and pulmonic artery areas at the end of systole in cases 1 and 2.

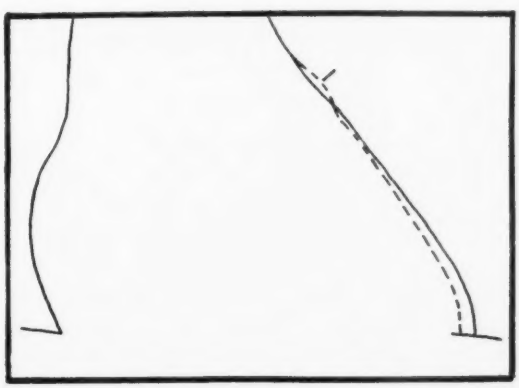

Chart 2.—Cardiac outline of case 3. The straight line indicates the end of diastole; the broken line, the end of systole. Note the limited motion of the left auricular area.

Two consecutive cycles of case 2 and two cycles from different records of case 3 are shown in charts 3 and 4, to give an idea of the variation from cycle to cycle in a given case.

In chart 5, graphic records of the motion of the left auricle, during systole and diastole in cases 1, 2 and 3 are compared with a correspond-

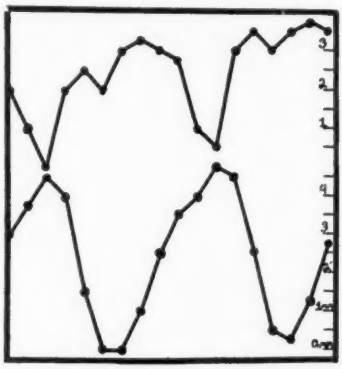

Chart 3.—Two consecutive cycles of case 2, the upper curve showing motion of the left auricle, the lower curve, apical movement. Lateral movement is shown by upward swing of the curves. Each point represents one film or a duration of one-fifteenth second.

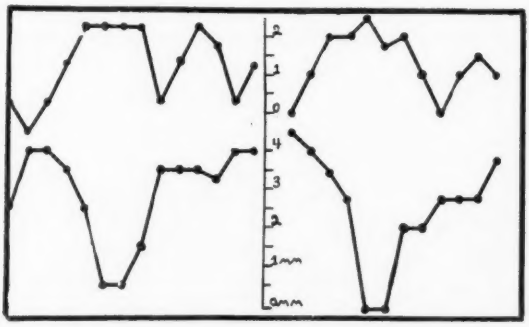

Chart 4 (case 3).—Two cycles, from two sets of films, showing, as in chart 3, auricular and apical movement.

ing record from the normal subject. It is immediately apparent from these curves that the auricular motion in cases of disease of the mitral valve is different from the normal. The motion of the auricle in case 3 is more like the normal than the other two, although clinically this patient presents the most typical case of stenosis of the mitral orifice. The early diastolic collapse, however, is less marked, and the mid-

diastolic distention and late diastolic (auricular systolic) emptying much more marked than in the normal.

The most striking curves are those in cases 1 and 2, which, except for the difference in rate, are almost alike. In them the auricle does not empty rapidly in early diastole, but on the contrary continues to expand during the first one-fifteenth of a second. It then has an initially slow but increasingly rapid emptying, without any middiastolic "shoulder," and with only the increase in rate of late diastolic emptying to indicate the effect of auricular systole. With the onset of ventricular systole the auricle suddenly dilates, and then, after falling back slightly, continues a slower dilatation into early diastole.

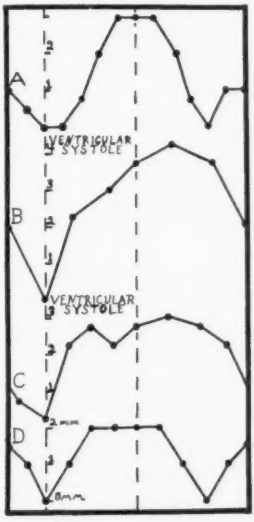

Chart 5.—Schema comparing auricular motion of the normal heart (*A*), with the motion of the auricles of cases 1, 2 and 3 of disease of the mitral valve (*B*, *C* and *D*). The initial downstroke presumably represents the motion during auricular systole, and the time relationships of the curves of the abnormal hearts are distorted so as to give systole and diastole the same relative duration as the normal, one complete cycle being given. Each point represents one film or a duration of one-fifteenth of a second. *B* and *C* show the continuation of auricular filling into early diastole in the two cases of regurgitation, *D* the relatively prominent mid-diastolic filling and presystolic discharge in pure mitral stenosis in case 3.

COMMENT

Our observations, particularly with regard to motion in the left auricular area, indicate that important data can be obtained from careful measurement of the roentgenograms of the diseased heart obtained with Ruggles' roentgen cinematograph. The value of this method of study-

ing the effect of valvular lesions will be greatly increased when satisfactory films of oblique projections can be taken, and this applies particularly to mitral lesions. Comparison of the motion of the left auricle with the size and shape of the mitral opening may be expected to shed great light on the vexed problem of mitral regurgitation, but this will be possible only when cases can be studied during the last months of the patient's life. Whether the roentgen cinematograph is necessary for these studies or whether satisfactory curves can be obtained more easily by the slit and moving film method of Gött and Rosenthal [4] has not been determined. The advantage of the cinematographic method is that it enables one to select any number of points in the cardiac outline for graphic analysis.

We can present no evidence with which to support our belief that the final emptying of the auricle is due to auricular systole, except that the time relationship of the final inward motion in the normal person and in case 3 corresponds approximately with that of auricular systole. Simultaneous electrocardiograms, on which the moment of each exposure is automatically recorded, should cast more light on this, as should also the form of the auricular curve of heart border motion in cases of auricular fibrillation without valvular lesion.

In case 3 the form of the auricular motion curve points definitely to mitral stenosis. The diminished rate and extent of early diastolic emptying and the great accentuation of that part of the curve supposedly due to auricular systole are to be expected with advanced anatomic stenosis, practically without regurgitation. Classic physical and electrocardiographic changes were found in this patient, but the teleroentgenogram was hardly more than suggestive of a mitral lesion, and there was no such bulging of the left auricular area as in cases 1 and 2. Of the latter cases, the first had a murmur audible throughout diastole, the second only in late diastole; in both the murmur was sharply localized near the apex and unaccompanied by a thrill. These observations are usually considered sufficient for the diagnosis of mitral stenosis, but this is not borne out by the roentgenograms of the heart. In both instances auricular movement is rapid and marked, and the rapid dilatation in early systole points definitely to regurgitation. This is further borne out by the sequence of events in diastole, the absence of any evidence of auricular systole except the increase in rate of mesad motion in late diastole, and the fact that dilatation continues for one-fifteenth of a second into early diastole. The latter finding was a puzzle to us at first.

4. Gött, T. H., and Rosenthal, J.: Ueber ein Verfahren zur Darstellung der Herzbewegung mittels Röntgenstrahlen (Röntgenkymographie) München. med. Wchnschr. **59**:2033, 1912.

but agrees exactly with Wiggers and Feil's [5] demonstration in animal experiment that mitral regurgitation continues from 0.08 to 0.09 seconds after the end of systole. The only evidence of mitral stenosis in the auricular movement of cases 1 and 2 was the relatively slow collapse when diastolic emptying had commenced. This is at once apparent on comparing these curves with the normal or with curves of auricular volume in experimental mitral insufficiency.[5]

In the analysis of the normal curve we had suggested [2] that one reason that the upper part of the ventricle moved mesad while the apex moved laterad in early diastole might be that the blood, streaming in along the long axis of the heart, might push out the apex and hence cause a narrowing of the upper part of the ventricle. In case 2 a similar phenomenon seems to be due, in part at least, to diastolic relaxation of the ventricle, the apex falling away from the base and the lateral wall inward as the contraction dies out, but this occurs while the auricle is still dilating.

We recognize that from one normal and three abnormal cases only tentative conclusions can be drawn. Further work will undoubtedly show wide variations in normal as well as in diseased hearts; yet there seems to be no doubt that a study of the curves of heart border motion offers a relatively accurate means of distinguishing mitral regurgitation from mitral stenosis, these terms being taken in the physiologic sense. Such a distinction has lapsed in some clinics, largely as a reaction to the former practice of making too frequent and often erroneous diagnoses of mitral regurgitation merely on the basis of a systolic murmur. Those who have led this reaction point out that practically all organic mitral lesions result in a narrowing of the orifice, and that regurgitation in the presence of organic stenosis of whatever degree had better be ignored. Elsewhere, and particularly in Europe, the diagnosis of mitral regurgitation is seriously considered in patients with myocardial failure and a prominent auricular area in the left border of the heart, whether determined by the roentgen ray or by percussion, and in patients with rheumatic valvular disease with loud systolic murmurs. Sprague and White [6] have recently discussed the entire question. It is their belief that mitral insufficiency can be diagnosed from the history and physical observations, and is a less serious lesion than stenosis. Our observations tend to give weight to Balfour's view [7] that mitral regurgitation has a definite character, and that "the only unequivocal proof of the existence of

5. Wiggers, C. J., and Feil, H.: The Cardiodynamics of Mitral Insufficiency, Heart. **9**:149, 1921.

6. Sprague, H. B., and White, P. D.: A Comparative Study of "Rheumatic" Mitral Regurgitation and Mitral Stenosis, Am. Heart J. **1**:629 (June) 1926.

7. Balfour, G. W.: Clinical Lectures on Diseases of the Heart and Aorta. London, 1876, p. 154.

actual disease of the mitral valves is the determination of the existence of an auricular-systolic, or as it is commonly termed, a presystolic murmur." He held, and we believe that it is a safe guide for the present time, that this is as true of mitral regurgitation (due to disease of the valve itself) as of mitral stenosis.

Clinical and experimental evidence is being advanced to prove the seriousness of mitral regurgitation. Gussenbauer [8] described a case of mitral insufficiency due to a gunshot in which an oval hole in the anterior mitral leaflet, not more than two-thirds the area of the aortic orifice, led to heart failure, auricular fibrillation and death in ten years. This patient did not have a syphilitic or rheumatic myocarditis, and there was an extraordinary dilatation of the left auricle. This shows the seriousness of such a lesion.[9] Of 147 patients with mitral stenosis whose hearts were examined at autopsy and reported on by Cabot,[9] eighty had mitral orifices of greater circumference than the traumatic aperture in Gussenbauer's case. It is noteworthy that our patient with pure mitral stenosis did not have any significant prominence of the auricular region, while the two patients in whom regurgitation was preponderant had the classic "mitral configuration." Experimentally, Allan [11] has shown recently how easily mitral stenosis is compensated until the aperture is reduced to minute size, and what a comparatively large load is put on the heart by regurgitation.

The importance of developing accurate clinical criteria for the differential diagnosis of mitral stenosis and mitral regurgitation is apparent. This is of utmost importance to those who attempt operation on the heart, for it will enable them to spare from operation patients whose lesions mainly produce regurgitation. The difficulty has been discussed by Souttar,[12] whose patient had a mitral orifice which easily admitted a finger.

SUMMARY

A study of the curves of heart border motion constructed from films taken with Ruggles' roentgen cinematograph shows that in mitral disease the motion of the auricular region is of great significance.

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8. Gussenbauer, R.: Ein Fall von traumatischer Mitral insuffizienz, Wien. med. Wchnschr. **75**:134 (Jan. 10) 1925.

9. Adam, A.: Ueber die traumatischen Veränderungen gesunder Klappen des Herzens, Ztschr. f. Kreislaufforsch. **9**:313 (May) 1927.

10. Cabot, R.: Facts on the Heart, Philadelphia, W. B. Saunders Company, 1926, p. 76.

11. Allan, G. A.: A Schema of the Circulation with Experiments to Determine the Additional Load on the Apparatus Produced by Conditions Representing Valvular Lesions, Heart. **12**: 181, 1926.

12. Souttar, H. S.: Surgical Treatment of Mitral Stenosis, Brit. M. J. **2**:603 (Oct. 3) 1925.

A patient with a marked thrill and loud diastolic murmur, but slight systolic murmur, gave curves which are interpreted as those of mitral stenosis.

Two patients with loud systolic and soft localized diastolic murmurs gave curves which apparently indicate the preponderance of regurgitation. In one of these, autopsy (eight months after our studies) revealed a mitral orifice with a circumference of 7 cm. held open by a scarred and calcified margin.

MATERIAL FROM LYMPH NODES OF MAN

I. METHOD TO OBTAIN MATERIAL BY PUNCTURE OF LYMPH NODES FOR STUDY WITH SUPRAVITAL AND FIXED STAINS*

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This paper deals with a method for obtaining living tissue from lymph nodes and tumors, and emphasizes the importance of studying living cells in pathologic processes. The results obtained from the study of abnormal lymph nodes by the application of these methods are discussed in a separate communication.[1]

Lymph nodes of patients usually are studied for diagnosis by ordinary pathologic technic after their surgical removal. Biopsy is not a procedure that can be repeated readily, and thus in following the development of disease processes, or the effect of therapy, it is not a satisfactory way of obtaining tissue for pathologic study. Furthermore, biopsy is refused by many patients, and is frequently difficult and at times impossible to perform with safety. Moreover, the usual technic for accurate diagnosis from the tissue obtained by biopsy requires several days. For these reasons material has been obtained from lymph nodes by puncture. This procedure has none of the disadvantages of biopsy, is simple, and seldom is objected to by patients, probably because they have no apprehension concerning the use of a needle such as they have concerning a procedure involving the use of the surgeon's knife. Puncture can be performed repeatedly and safely in many cases in which surgical removal of the node would be unwise or difficult. The cellular material obtained can be studied both in fixed and in fresh preparations. The living cells supravitally stained have furnished new and useful information. By such procedures much may be learned and a diagnosis probably made in less than half an hour, in contrast to several days required by the technic for the usual pathologic diagnosis. Whether the knowledge obtained by studying cellular material from nodes will be as serviceable as that deduced from examining sections of a lymph node, can be decided only after many comparisons and observations have been made.

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* From the Medical Service of Collis P. Huntington Memorial Hospital of Harvard University.

1. Forkner, C. E.: Studies on Living and Fixed Cells Withdrawn from Lymph nodes of Man, to be published.

HISTORY OF PUNCTURE OF LYMPH NODES

Puncture of lymph nodes seems to have been confined especially to schools of tropical medicine. Aspiration of material from nodes apparently was first undertaken by Grieg and Gray in 1904,[2] in order to isolate the etiologic agent of trypanosomiasis. Since then, numerous investigators have aspirated lymph nodes with a syringe and needle to recover the organisms of certain tropical diseases and of syphilis. None of those physicians, however, has reported on the cellular material obtained. Ward, in 1912,[3] suggested that the appearance of cells obtained by puncture of nodes might aid in the diagnosis of the various forms of lymphoblastoma. He was aware of one such case in which the procedure was performed without ill effect. Guthrie, in 1921,[4] made a serious attempt to study the tissue cells obtained by aspiration from patients' lymph nodes. He has described the appearance in fixed stained preparations of the cells he withdrew in different forms of lymphoblastoma and conditions simulating them. From the material obtained he was able to make the diagnosis of Hodgkin's disease—a type of lymphoblastoma according to some authorities. Guthrie's important report is of a preliminary nature, however, and leaves many matters inconclusive.

Guthrie [4] punctured lymph nodes with a 21 gage needle on the end of a syringe, rotated it once, and then by negative pressure withdrew into the syringe as much material as possible. He says that in many cases a scant amount of material was obtained because of the firm consistency of the tissue. This difficulty was only too apparent when I began puncturing nodes by this method, and sometimes tissue could not be obtained, or the amount was insufficient for adequate study. I have almost invariably been able to obtain enough material by employing a barbed dental broach passed through the lumen of a small needle inserted into the substance of the node. Large numbers of living cells are withdrawn easily on the barbs. Other methods of puncture were tried, but the following was adopted as the most satisfactory:

TECHNIC

The procedure which has been developed and utilized in studies on lymph nodes is as follows: The skin overlying the structure to be punctured is cleaned surgically by scrubbing with soap, followed by the application of ether and iodine, or by mercuric chloride or picric acid solutions. About 0.5 cc. of procaine hydrochloride in solution is infiltrated through a small hypodermic needle into the skin. A 17 or 18 gage sterile needle about 3.5 cm. in length is then inserted

2. Grieg, E. D. W., and Gray, A. C. H.: Brit. M. J. **1**:1252, 1904. Lymphatic Glands in Sleeping Sickness, Lancet **1**:1570, 1904.

3. Ward, G. R.: Bedside Haematology, Philadelphia, W. B. Saunders Company, 1914, p. 129.

4. Guthrie, C. G.: Gland Puncture as a Diagnostic Measure, Bull. Johns Hopkins Hosp. **32**:266, 1921.

through the anesthetized area until the point pierces the capsule of the lymph node or diseased tissue. The exact position of the needle point is easily determined by the resistance it encounters and by the movability of the mass. After the needle has been passed just within the tissue from which material is desired, the needle is steadied and a sterile coarse dental broach[5] is passed through the lumen. These broaches are constructed so that they enter the tissue like a needle, but come out like a fish hook, carrying on the barbs numerous elements of living tissue. Smears of the material obtained are then quickly made on glass coverslips. One puncture of a tumor or node yields sufficient material for several smears. The more cellular the tissue the more material is obtained from which cultures as well as smears may be made. After sufficient material has been obtained by inserting one or more broaches, the needle is withdrawn and a small dry sterile dressing applied. The procedure seems to be no more painful than puncturing a vein in the arm for the withdrawal of blood, and there has been no difficulty in making repeated punctures of lymph nodes on the same patients.

VALUE OF STUDIES ON LIVING CELLS

The particular studies made on the material collected by this type of puncture have been observations of fixed smears stained with Wright's stain and supravitally stained preparations. Heretofore, information has not been reported concerning living cells from human lymph nodes examined by the aid of supravital stains. Webster[6] and Lewis and Webster[7] made studies on the lymphocytes in tissue cultures of normal and pathologic lymph nodes. They observed that lymphocytes were capable of migrating into the plasma clot from "explanted pieces of lymph-nodes." Webster[6] believed that evidence of ameboid activity on the part of the lymphocytes was indicative of a rapidly fatal course. These authors have not reported any studies concerning the reaction of living cells of lymph nodes to vital dyes. It would appear to be possible to acquire physiologic as well as histologic knowledge of a new sort by this means. For example, observations may be made on ameboid activity, phagocytic power, behavior to altered environment, resistance or susceptibility to staining and other characteristics. It is not possible adequately to obtain such information from observations on fixed tissues. Thus a definite opinion can be arrived at concerning the age, stage of development and activity of the cells.

The ordinary histologic methods require that tissues be subjected to rigorous measures, which not only obscure the physiologic processes of

5. The dental broaches are obtainable from almost any dental supply company. Broaches with aluminum handles are the most satisfactory. For these studies the "Dayton Dental Broach-Improved-Coarse-Style Number 1" has been used.

6. Webster, L. T.: Lympho-sarcoma, Lymphatic Leukemia, Leukosarcoma, Hodgkin's Disease, Bull. Johns Hopkins Hosp. **31**:458, 1920.

7. Lewis, W. H., and Webster, L. T.: Migration of Lymphocytes in Plasma Cultures of Human Lymph nodes, J. Exper. Med. **33**:261, 1921.

cells but also produce artefacts. If one contrasts the study of living cells with that of dead cells, the protoplasm of which has been coagulated by strong fixing agents, altered by dehydration and shrinking in alcohol and subjected to abnormal processes in embedding and then cut at various angles, the value of studying living cells becomes apparent. Like any procedure, practice and experience is required to make accurate and reliable observations on living supravitally stained cells.

The behavior of blood cells to vital dyes was studied as long ago as 1902 by Rosin and Bibergeil,[8] but it is the work of Evans and Scott,[9] Simpson [10] and Sabin [11] which has developed efficiently the so-called supravital method. These workers and their pupils have shown the value of the method and contributed much information concerning living blood cells. Details of technic are given by Simpson [10] and Sabin.[11] I shall give a brief description to indicate its nature and the modifications found useful for studying lymph node material.

A thin film of dye in alcoholic solution is smeared across the surface of a previously cleaned, polished and warmed glass slide. This is done by dipping the end of another slide, with which the smear is made on the former one, into a fresh mixture (10 drops) of equal parts of 0.5 per cent alcoholic solution of neutral red and a 0.1 per cent alcoholic solution of janus green. Solutions of the dyes are kept on hand, made up in 95 per cent alcohol. The dyes are not mixed until they are ready for use, because a mixture is not a stable preparation. Solutions of varying concentrations should be employed for making the dye films until one has gained proficiency in estimating the optimum concentration desired. Emphasis should be placed on staining the cells as lightly as is consistent to obtain good differentiation of the structures they contain. If the dyes are employed in concentrated solutions, the nuclei will be stained and the cells quickly killed. The glassware must be scrupulously clean because cells are delicate structures, and any acid, alkali or the like will injure their membranes and markedly alter their physiologic behavior.

METHOD MODIFIED FOR TISSUE CELLS

In the study of cells from blood, a free-flowing drop of blood is procured on the under surface of a glass coverslip, and this is allowed to fall gently on the slide prepared with the dye. In the case of blood, the plasma takes up some of the dye and acts as a medium in which the cells maintain their activity in suspension. However, material obtained from nodes, especially if fibrosed, often has

8. Rosin, H., and Bibergeil, E.: Ergebnisse vitaler Blutfärbung, Deutsche med. Wchnschr. **27**: 41, 1902. Rosin, H.: Das Verholten der Leucocyten bei den vitalen Blutfärbung, Virchows Arch. f. path. Anat. **178**:478, 1904.

9. Evans, H. M., and Scott, K. T.: On the Segregation of Macrophage and Fibroblast Cells by Means of Vital Acid Dyes and on the Cause of the Differential Effect of These Substances, Anat. Record **16**:148, 1919.

10. Simpson, M. E.: Vital Staining of Human Blood with Special Reference to the Separation of the Monocytes, Uni. Calif. Pub. Anat. **1**:1, 1921

11. Sabin, F. R.: Studies on Living Human Blood Cells, Bull. Johns Hopkins Hosp. **34**:277, 1923.

insufficient fluid to permit cells to be properly suspended. The addition of physiologic sodium chloride alters the staining qualities and behavior of the cells, and is not ideal for this purpose. As a fluid in which to suspend the cells one can use the patient's serum or that from a person belonging to iso-agglutination group I (Moss classification) whose serum does not agglutinate red blood corpuscles. I have also used successfully sterile clear fluid obtained from ovarian cysts. It is essential to have a fluid medium in which to suspend the cells immediately after their removal from the body in order to prevent desiccation and to promote a uniform staining reaction.

The staining properties of blood cells and their physiologic response to dyes are excellently described by Simpson [10] and Sabin,[11] and for such information reference should be made to their articles. Suffice it to say here that janus green and neutral red are relatively nontoxic dyes; the former in dilute concentrations is a specific stain for mitochondria, and the latter a specific one for cytoplasmic structures. Their application to tissue cells is discussed in the subsequent article.

INDICATIONS AND CONTRAINDICATIONS FOR PUNCTURE

The safety of puncturing lymph nodes of various sorts has been considered carefully, and it appears that this procedure is to be looked on as harmless if used with discretion. One must be acquainted with the anatomy of the given region before attempting to make a puncture. About forty punctures have been made in nodes proved by sections to be diseased with various types of lymphoblastoma, metastatic cancer and other tumors, myelogenous leukemia, tuberculosis and simple hyperplasia. Local or general dissemination of a tumor or spread of disease which could be attributed to puncture has not been observed. There is no evidence that infection, hemorrhage into nodes or significant discomfort has occurred. Biopsy in some cases of lymphoblastoma and metastatic cancer may be followed by a rapid spread of the disease, and perhaps eventually this may be shown to result from puncture. Puncture, however, requires less manipulation and trauma than surgical removal of a lymph node.

Puncture of lymph nodes seems contraindicated in cases of probable or definite malignant disease when the possibility of cure by total removal of the pathologic tissue exists. It is best not to injure such neoplasms. Another contraindication is immediate proximity of the structure to be punctured to a large artery. The exact location of the needle point can usually be accurately ascertained, and thus one need not be alarmed if an artery is nearby.

Puncture of a node does not interfere with its subsequent removal. This sequence of events has taken place in several of the cases studied in order to correlate the results obtained from material collected by puncture with those recorded from observations of sections of nodes

prepared by the usual pathologic technic. Such correlations in many more cases than I have studied are essential before a final opinion concerning the value of puncture of lymph nodes can be decided. The procedures referred to, however, appear to be of some service as indicated in my second paper.

SUMMARY

Puncture of lymph nodes by means of a hollow needle through which a dental broach is passed is a new, apparently harmless and satisfactory way to obtain sufficient material from patients for study.

The value of studying the living cells from diseased lymph nodes by supravital staining is emphasized.

DIABETES MELLITUS

INCIDENCE AND CERTAIN ETIOLOGIC FACTORS *

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AND
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Great interest has been manifested in various problems bearing on the etiology of diabetes mellitus, especially during the last few years. This is due largely to the menace of the disease as revealed by the increase in its incidence as well as in its mortality rate, at least until the time of the introduction of insulin.[1]

The observations of Emerson and Larimore [2] have definitely proved that there has been a progressive and actual increase in the death rate from diabetes in New York, as well as in other larger cities, between the years 1860 and 1923. They direct attention to the fact that an increase in the death rate from a certain disease may be considered as pointing to an actual increase in its incidence, especially during a period in which clinical diagnosis as well as treatment have progressed to such an extent as to make possible early recognition of the disease; arrest in early cases and postponement of death when cure cannot be expected. This, they point out, can be assumed to be true of diabetes, since great advances have been made in both diagnosis and treatment.

Table 1 presents the average death rate from diabetes per 100,000 population for Baltimore, Philadelphia and New York for twenty years in five year periods. It is evident that there was a progressive increase in the mortality rate of the disease until 1923; it was greater in Baltimore than in Philadelphia, and greater in Baltimore than in New York since 1910.

Table 2, furnished through the courtesy of Dr. C. Hampson Jones, commissioner of health of Baltimore, demonstrates that notwithstanding

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*Presented at the Meeting of the American Gastro-Enterological Association at Atlantic City, May 3, 1927.

*From the Gastro-Enterological Clinic of the Department of Medicine of the University of Maryland, Baltimore.

1. The Metropolitan Life Insurance Company ascribes the lowering in the death rate from diabetes among their industrial policy holders during the last half of 1923 and the first quarter of 1924, to the successful and widespread use of insulin (Statistical Bulletin Metropolitan Life Insurance Company, vol. 5, no. 2, [Feb.] 1924).

2. Emerson and Larimore: Diabetes Mellitus, Arch. Int. Med. **34:** 585 (Nov.) 1924.

a fall from 21.01 to 14.41 in the general death rate in Baltimore from 1900 to 1924 (i. e., deaths from all causes for each 1,000 of the population), there was a rise from 6.9 to 24.2 in the death rate from diabetes for each 100,000 of population. The death rate per 100,000 from this dis-

TABLE 1.—*Average Death Rate from Diabetes per 100,000 Population for Baltimore, Philadelphia and New York for Twenty Years, According to Five Year Periods* **

	1900 to 1904	1905 to 1909	1910 to 1914	1915 to 1919	1920 to 1924
Baltimore	9.3	12.4	16.8	20.3	23.8
Philadelphia	9.1	11.2	15.5	15.0	17.0
New York	12.3	14.8	17.0	19.8	21.4

* The Philadelphia and New York figures are taken from Anders and Jameson.[3]

TABLE 2.—*Deaths and Death Rates from all Causes and from Diabetes in Baltimore, from 1900 to 1924*

Year	Population	Total Deaths All Causes	Crude Death Rate per 1,000	Deaths from Diabetes			Diabetes Death Rate per 100,000	Percentage Diabetes Deaths of All Deaths	Age Groups					
				Total	Male	Female			0 to 19		20 to 44		45	
									M	F	M	F	M	F
1900	509,375	10,760	21.01	35	..	...	6.9	0.33	..	..	..	..	..	...
1901	514,390	10,479	20.37	49	..	...	9.5	0.47	..	..	..	..	..	...
1902	519,406	10,253	19.74	46	..	...	8.9	0.45	..	..	..	..	..	...
1903	524,421	10,141	19.34	55	..	...	10.5	0.54	..	..	..	..	..	...
1904	529,437	10,818	20.43	57	..	...	10.8	0.53	..	..	..	..	..	...
1905	534,452	10,695	20.01	56	..	...	10.5	0.52	..	..	..	..	..	...
1906	539,468	10,753	19.93	72	..	...	13.3	0.67	..	..	..	..	..	...
1907	544,483	11,190	20.55	83	..	...	15.2	0.74	..	..	..	..	..	...
1908	549,499	10,435	18.99	61	..	...	11.1	0.58	..	..	..	..	..	...
1909	554,514	10,376	18.71	67	..	...	12.1	0.65	..	..	..	..	..	...
1910	559,530	10,753	19.22	93	..	...	16.6	0.86	..	..	..	..	..	...
1911	564,545	10,404	18.43	98	..	...	17.4	0.94	..	..	..	..	..	...
1912	569,560	10,441	18.33	88	..	...	15.5	0.84	..	..	..	..	..	...
1913	574,576	10,679	18.59	87	..	...	15.1	0.82	..	..	..	..	..	...
1914	579,592	10,551	18.20	115	..	...	19.8	1.09	..	..	..	..	..	...
1915	584,607	10,002	17.11	109	45	64	18.6	1.09	3	1	8	7	34	56
1916	589,623	10,719	18.18	122	54	68	20.7	1.14	2	4	10	7	42	57
1917	594,638	11,364	19.11	124	56	68	20.9	1.09	3	2	10	7	43	59
1918	599,653	16,032	26.74	119	48	71	19.8	0.74	2	3	9	10	37	58
1919	669,981	11,434	17.07	145	54	91	21.6	1.27	3	1	7	16	44	74
1920	740,172	11,356	15.34	155	64	91	20.9	1.36	2	2	11	11	51	78
1921	752,865	10,380	13.78	169	58	111	22.4	1.63	1	4	7	12	50	95
1922	762,222	10,824	14.20	200	71	129	26.2	1.85	5	5	5	8	61	116
1923	773,580	11.588	14.98	195	82	113	25.2	1.68	3	2	9	9	70	102
1924	784,938	11,310	14.41	190	59	131	24.2	1.68	1	3	11	8	47	120

ease was, therefore, more than three times greater in 1924 than in 1900. It will also be noted that the number of deaths from diabetes in 1924 (190) exceeded those of 1900 (35) more than five times, while the population of the former year was but little over one and a half times larger than that of the latter year. A more marked increase was noted

by Anders and Jameson [3] in their mortality figures for Philadelphia. As Emerson and Larimore have pointed out, while this increase in the incidence of the death rate has included persons of all ages, it has been marked in women at all ages and in men over 45. Our observations have confirmed these facts.

According to the statistics furnished by the Bureau of Census [4] of Washington, the death rates from diabetes show a steady increase upward between 1910 and 1920. Most of this increase is due to the higher rates above the age of 45, the menace of the disease being more especially noted among women over that age.

Joslin [5] attributes this increase in diabetes to a number of factors: (1) to a greater accuracy of vital statistics; (2) to urinary examinations more frequently performed as a routine; (3) to the general increase in longevity, which should necessarily add to the incidence of this disease; (4) to life insurance examinations and statistics through which the recognition of new cases has more frequently been made possible, and (5) to the frequency by decades at which the onset of this disease occurs at the present time, which has likewise made comparatively greater the increase of cases in the first decade as compared with the increase shown in former statistics. Among the interesting problems associated with the etiology of diabetes is the well recognized observation of the greater hazard of this disease among the Jewish as compared with the non-Jewish population. The disease has been significantly termed "Juden-Krankheit"; because of the great prevalence of this condition among the Jews.

As the tabulations in New York and in other cities do not include the religious belief of the deceased (not noted in the death certificates), it is impossible, according to Emerson and Larimore, to obtain a wholly reliable basis for the calculation of the death rates by races. On the other hand, these observers point to an interesting correlation in New York City and Boston between the increase in the percentage of Jews in the population over a period of years and the increase in the death rates from diabetes for the same years in the two cities.

According to Emerson and Larimore, it would seem, "to be more than an unrelated coincidence that a city like New York with more than half of all of the Jews of the country, has shown in the last thirty years a greater increase in diabetes death rates than any other large city, and a percentage increase of rate running parallel with the percentage of difference in the Jewish element in the population."

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3. Anders and Jameson: Am. J. M. Sc. **170**:313 (Sept.) 1925.

4. Department of Commerce, Bureau of Census Mortality Rates, 1910-1920, 1923, p. 87.

5. Joslin: Treatment of Diabetes Mellitus, Philadelphia, Lea & Febiger, 1923, p. 121.

The testimony of many observers tends to corroborate the fact that diabetes occurs more frequently among the Jews. According to Morrison,[6] there were 1,775 deaths from diabetes in Boston from 1895 to 1913. Of these, 127 occurred among the Jews, among whom the total number of deaths was 6,936, while 1,648 occurred among the Christians, among whom the total number of deaths was 222,532.

The ratio of the number of deaths from diabetes to the total number of deaths from all causes is 0.018 among Jews and 0.007 persons who are not among Jews; diabetes has, therefore, been two and one half times as frequent a cause of death among the Jews as among the rest of the population.

The reports abroad of Auerbach,[7] Wallach,[8] Singer,[9] Frerichs,[10] Kulz [11] and Van Noorden [12] indicate the greater incidence of diabetes in the Jewish race as compared to other races. In this country, Stern,[13] Rudish and Aronson,[14] Billings,[15] and Joslin [16] have arrived at a similar conclusion.

The cause of the special susceptibility of the Jews to this disease has not yet been satisfactorily determined.

According to Emerson and Larimore, "sedentary occupations, unsuitable dietary habits, luxurious living, disinclination to indulge in physical exercise, a marked tendency to obesity in men and women among the Jews; particularly after 45 years of age, may well be more definite etiologic factors in the apparent excess of diabetes death rates among the Semitic people than their race."

S. Solis Cohen [17] attributes the disproportionate prevalence of diabetes, first, to unsuitable dietetic habits brought over by the Jewish immigrants from Russia, which to a great degree accounts for the coincident obesity, and secondly, to the emotional disturbances due to centuries of persecution which produced an autonomic endocrine inbalance.

6. Morrison: Boston M. & S. J. **175**:55 (July 13) 1916.
7. Auerbach: Ztschr. Demogr. Stat. Juden, 1908, p. 164; quoted by Fishberg: The Jews; A Study of Race and Environment, 1911, p. 298.
8. Wallach: Deutsche med. Wchnschr. **19**:779, 1893.
9. Singer: Krankheitslehre der Juden, Leipzig, 1904, p. 81.
10. Frerichs: Ueber den Diabetes, Berlin, 1884, p. 185.
11. Kulz: Klinische Erfahrungen ueber Diabetes Mellitus, Jena, 1899, p. 2.
12. Van Noorden: Zuckerkrankheit und Ihre Behandlung, Ed. 6, Berlin, 1912, p. 54.
13. Stern: M. Rec. **58**:766 (Nov. 17) 1900.
14. Rudish and Aronson: Mt. Sinai Hosp. Rep. **11**:26, 1899-1900.
15. Billings: United States Census Bulletin, Vital Statistics of the Jews in the United States **19**:15 (Dec. 30) 1890.
16. Joslin: Diabetic Problem of Today, J. A. M. A. **83**:727 (Sept. 6) 1924.
17. Cohen, S. Solis, in discussion on Anders and Jameson: J. A. M. A. **84**: 1776 (June 6) 1925.

Von Noorden [18] believes that the frequent racial intermixture of Jewish with other blood is the cause of the excessive prevalence of diabetes among the Jews, while others attribute it to the frequency of consanguineous marriages. In analyzing the predominant factors associated with the etiology of diabetes, Joslin [19] calls attention to the fact that many of these, especially observed in the Jewish race, such as the occurrence of this disease in the conjugal form, its presence among the wealthier classes, its hereditary tendency, its occurrence in mental workers in contradistinction to physical workers and as a sequel to infectious diseases, can all be readily accounted for on the basis of an associated obesity.

The relationship between diabetes and obesity has been exhaustively studied by Joslin.[19] A study of the weight of his adult diabetic Jewish patients before the disease manifested itself indicates that they were more obese than the patients who were not Jewish. Joslin says that "85 per cent of the Jewish patients were overweight in contrast to 70 per cent of a mixed series of 1,000 Gentiles; 4.7 per cent of these were underweight in contrast to 12.2 per cent of the non-Jewish patients." According to Joslin, if diabetes were especially characteristic of the Jewish race, it would be noted equally from youth to old age; however, in comparing the age incidence of this disease in 2,611 of his patients of all nationalities, he noted that instead of a parallel incidence through all the life periods, it was less common among the Jews in the first two decades of life, but more frequent during the fourth and fifth decades. The Jewish child is, therefore, no more likely to become a victim of this disease than the child who is not Jewish. On the other hand, the Jewish patient contracts the disease, not because of his race, but because of obesity.

Joslin also calls attention to the significant fact that the death rates from diabetes are essentially the same in New York and in Boston, notwithstanding the fact that three tenths of the population of New York is Jewish and but one tenth of that of Boston. On the other hand, in Brookline, in which the Jewish population is scarcely proportionately larger than in Boston, the death rate is double, undoubtedly due to the proportionately greater number of older, wealthier and more obese Jews.

In this connection the studies of Anders and Jameson [20] are of especial interest; they made the observation that the average percentage of overweight is higher among the Jewish women with diabetes than among the men, and that the incidence of diabetes among the obese Jews is higher than among the obese Gentiles, the ratio being 12 to 8.

18. Von Noorden: Berl. klin. Wchnschr. **37**:1117, 1900.

19. Joslin: Prevention of Diabetes, J. A. M. A. **76**:79 (Jan. 8) 1921.

20. Anders and Jameson: (Footnote 3, p. 319).

The relation of obesity to diabetes becomes still further evident in the study of sugar tolerance curves in obese persons. According to John,[21] the maximum increase in the blood sugar concentration in the nondiabetic person occurs promptly following the ingestion of 100 Gm. of dextrose—in 58 per cent of cases in one-half hour; in 33 per cent, in one hour; and in 8 per cent, in two hours. In diabetic persons the rise in blood sugar concentration is slow, as is also the return to the normal level. In only 3.8 per cent of cases did the maximum rise appear one-half hour after the ingestion of the dextrose; in 15.4 per cent, it appeared at the end of one hour; in 54 per cent, at the end of two hours, and in 23 per cent, at the end of three hours. According to this observer, if the curve returns to the normal level in three hours following the ingestion of 100 Gm. of dextrose, the person is considered not diabetic, if more than three hours is required, he is considered diabetic. The cases in which the return to the normal level occurs in about three hours are recorded as being in the prediabetic stage.

Paullin and Sauls,[22] in a study of the blood sugar tolerance to dextrose, arrive at a somewhat similar conclusion in the study of twenty-six obese persons, of whom 57.6 per cent gave an abnormal response to the ingestion of dextrose. Six of the persons were classified by this method as having early cases of diabetes.

Beeler and Fitz [23] also observe that a certain group of obese persons present a curve of glycemia following the ingestion of 100 Gm. of dextrose resembling that of mild diabetes.

It is evident from these observations that a prediabetic stage may be recognized in a certain number of obese persons.

Following the observations of Paullin and Sauls, we have made a study of blood sugar tolerance to dextrose, noting especially the curve of glycemia, in forty-five obese Jews whose weight was compared with the standard weight of a person of the same age and height as noted in the table of the Association of Life Insurance Directors and Actuaries of America. The result of our observations are noted in table 3. Of the twenty-five persons, thirty-one, or 68 per cent, gave an abnormal response to the ingestion of the dextrose; of these, eight were found to have early diabetes. The abnormal response is indicated by an increase in sugar of over 0.17 per cent following the ingestion of dextrose; the curve for sugar is high and rather sustained, and a longer period is required for its return to normal; glycosuria is usually present.

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21. John: J. Metab. Research, **4**:286 (Sept.-Oct.) 1923; Am. J. M. Sc. **173**: 184 (Feb.) 1927.

22. Paullin and Sauls: South. Med. J. **15**:249 (April) 1922.

23. Beeler and Fitz: Glycemia, Glycuresis and Water Excretion in Obesity, Arch. Int. Med. **28**:804 (Dec.) 1921.

TABLE 3.—*Blood Sugar Tolerance Tests in Forty-five Obese Jews of from 12 to 67 Per Cent Overweight, Arranged According to the Percentage of Overweight*

No.	Name	Sex	Age	Weight	Pounds Overweight	Per Cent Overweight	Blood Sugar After 100 Gms. Dextrose					Urinary Examinations After 100 Gms. Dextrose					
							Fasting	½ Hour	1 Hour	2 Hours	3 Hours	Fasting	½ Hour	1 Hour	2 Hours	3 Hours	3 Hours
1	S. M.	♂	63	164	18	12	0.110	0.142	0.145	0.120	0.100	O	O	O	O	O	N
2	W. J.	♀	48	170	19	12	0.120	0.134	0.146	0.192	0.154	O	O	X	X	X	AP
3	F. H.	♂	60	185	20	12	0.098	0.146	0.148	0.121	0.102	O	O	O	O	O	N
4	K. M.	♂	61	195	21	12	0.096	0.126	0.148	0.176	0.114	O	X	X	O	O	A
5	F. A.	♂	59	202	23	12	0.102	0.128	0.162	0.184	0.110	O	X	X	X	O	A
6	W. R.	♂	51	212	24	12	0.104	0.132	0.168	0.174	0.152	O	X	X	X	O	A
7	K. R.	♂	47	186	22	13	0.100	0.154	0.148	0.114	0.100	O	O	O	O	O	N
8	P. J.	♀	60	192	22	13	0.112	0.148	0.148	0.110	0.110	O	O	O	O	O	N
9	F. E.	♂	61	194	26	13	0.110	0.152	0.156	0.124	0.112	O	O	O	O	O	N
10	G. H.	♂	66	220	26	13	0.121	0.162	0.184	0.148	0.134	O	O	X	X	O	A
11	W. I.	♂	54	159	20	14	0.114	0.142	0.176	0.125	0.102	O	O	X	X	O	A
12	K. M.	♂	54	218	28	15	0.104	0.136	0.148	0.120	0.114	O	O	O	O	O	N
13	S. L.	♀	68	186	44	16	0.111	0.146	0.158	0.122	0.100	O	O	O	O	O	N
14	W. S.	♂	39	212	30	16	0.104	0.152	0.194	0.154	0.112	O	O	X	X	O	A
15	G. J.	♂	57	209	34	19	0.120	0.152	0.150	0.112	0.110	O	O	O	O	O	N
16	T. R.	♀	57	186	32	20	0.160	0.240	0.246	0.200	0.150	O	O	O	X	O	A
17	W. T.	♂	59	185	33	20	0.142	0.196	0.220	0.212	0.148	O	O	X	X	O	A
18	S. F.	♀	58	222	37	20	0.124	0.260	0.245	0.250	0.134	O	X	X	X	O	A
19	W. B.	♂	63	200	36	22	0.095	0.150	0.144	0.110	0.100	O	O	O	O	O	N
20	K. L.	♂	54	178	32	22	0.112	0.148	0.198	0.210	0.124	O	O	X	X	O	A
21	J. T.	♂	62	204	39	23	0.142	0.154	0.184	0.284	0.292	O	O	X	X	X	AP
22	M. O.	♂	71	186	40	27	0.148	0.194	0.182	0.146	0.140	O	O	O	X	O	A
23	N. F.	♂	68	194	44	29	0.152	0.182	0.195	0.172	0.156	O	X	X	X	O	A
24	B. C.	♀	52	205	48	30	0.124	0.168	0.174	0.182	0.148	O	O	X	X	O	A
25	K. R.	♂	56	212	50	30	0.114	0.152	0.164	0.188	0.158	O	O	X	X	O	A
															trace		
26	T. F.	♂	59	172	42	32	0.098	0.150	0.144	0.124	0.110	O	O	O	X	O	N
27	O. L.	♂	65	192	49	34	0.124	0.184	0.216	0.175	0.122	O	O	X	X	O	A
28	P. O.	♂	75	172	46	36	0.116	0.168	0.184	0.170	0.112	O	O	O	X	O	A
29	F. M.	♂	69	186	50	36	0.109	0.178	0.176	0.162	0.118	O	O	X	X	O	A
30	J. K.	♀	59	192	52	37	0.120	0.154	0.186	0.180	0.134	O	O	X	X	O	A
31	T. B.	♂	62	168	48	40	0.138	0.186	0.198	0.216	0.202	O	X	X	X	X	AP
32	P. S.	♂	58	216	70	40	0.096	0.146	0.148	0.132	0.112	O	O	O	O	O	N
33	M. K.	♂	68	205	69	41	0.126	0.184	0.188	0.170	0.134	O	O	O	O	O	A
34	F. T.	♂	54	225	70	45	0.162	0.198	0.214	0.284	0.218	O	X	X	X	X	AP
35	S. L.	♂	58	175	56	47	0.098	0.168	0.184	0.188	0.130	O	O	X	X	O	A
36	B. P.	♀	53	179	58	48	0.112	0.134	0.146	0.144	0.100	O	O	O	O	O	N
37	S. F.	♂	74	204	67	48	0.128	0.138	0.186	0.180	0.132	O	O	X	X	O	A
38	N. D.	♂	67	220	72	48	0.122	0.164	0.198	0.192	0.148	O	O	X	X	O	A
39	W. C.	♂	59	228	74	48	0.144	0.188	0.222	0.254	0.298	O	X	X	X	X	AP
40	J. F.	♂	70	182	63	53	0.114	0.148	0.136	0.132	0.102	O	O	O	O	O	N
41	B. M.	♂	71	192	68	54	0.148	0.158	0.198	0.220	0.210	O	O	X	X	X	AP
42	K. T.	♀	62	178	67	60	0.154	0.194	0.228	0.254	0.216	O	X	X	X	X	AP
43	F. S.	♂	60	176	68	61	0.094	0.144	0.140	0.114	0.102	O	O	O	O	O	N
44	C. F.	♀	55	198	72	62	0.138	0.182	0.238	0.242	0.224	O	X	X	X	X	AP
45	K. M.	♀	66	174	70	67	0.128	0.164	0.232	0.214	0.148	O	O	O	X	O	A

In this table ♂ indicates male; ♀, female; N, normal response; A, abnormal response and AP, prediabetic response.

TABLE 4.—*Summary of Cases in Table 3, Arranged According to the Percentage of Overweight*

Percentage of Overweight	Number of Patients	Number of Normal Blood Sugar Responses	Number of Abnormal Blood Sugar Responses
10–19	15	8	7
20–29	8	1	7
30–39	7	1	6
40–49	9	2	7
50–59	2	1	1
60–69	4	1	3
Total	45	14	31

While the number of our cases is perhaps too small to warrant definite conclusions, nevertheless when one compares the response to the ingestion of dextrose among our obese Jewish patients with the result obtained by Paullin in his obese patients in general, there appears to be a considerable higher percentage of abnormal responses among the Jews than among persons who were not Jews: 68 per cent as compared to 57.6 per cent. This variation may be assumed to be further evidence in favor of the theory that there is a greater tendency among obese Jews to a lowered sugar tolerance and, consequently, to diabetes, than among non-Jewish persons.

Considerable attention has been directed to the progressive increase in the consumption of sugar during the past years as a factor in the production of obesity and, therefore, of diabetes. Riesman[24] ascribes the cause of the increase in diabetes to the greater per capita consumption of sugar added to an increase in sedentary habits.

According to Taylor,[25] 10 per cent of the population of the United States consumes over 4,000 calories daily without the corresponding necessary requirements for this amount, while a similar percentage of active workers consume less than 2,000 calories. He concludes that the work of prosperity increases food consumption while the idleness of hard times reduces it.

It is probable, as Emerson and Larimore point out, that "among certain racial, economic and age groups, prosperity tends to tempt them into food habits which exaggerate the usual discrepancy between the amount of food intake and the quantity needed for their respective heat, growth, repair and work requirements."

According to Taylor, with the annual addition of 100 pounds of sugar per capita to the other food, 500 calories a day alone are furnished in the average consumption of 3,600 calories of sugar. Emerson and Larimore furnish tables to illustrate that the rise or fall in the rate of consumption of sugar in the United States, Great Britain and France is followed by a similar rise or fall in the death rate from diabetes. This fact was especially noted during the period of the World War.

While it is evident that there is a tendency toward increase in the consumption of sugar, this fact alone cannot account for the increase in the incidence of diabetes. The increased consumption of sugar is simply an index to the tendency to the use of food in general far beyond the bodily requirements; this is especially true after middle life.

24. Riesman, quoted by Forchheimer: Therapeuses of Internal Diseases, New York, D. Appleton & Company, 1914, p. 718.

25. Taylor: J. Home Economics, **16**:55 (Feb.) 1924; Harvard Economic Review **2**:283 (April) 1924.

Taylor [26] says that:

Since we are consuming 100 pounds of sugar a year as compared with 75 pounds only a few years ago, this means we are consuming less of cereals, meats, fats or oils. When the dietary habits of overweight individuals are scrutinized, it will be observed that sugar is the food stuff gorged by some, starches by others, fats and oils by others, and meats by others. There is evidence tending to show that the level of average total caloric intake has risen, while the level of average physical exertions has declined; the combined result is an increase in average body weight after the age of 40.

Directly speaking, however, the average increase in body weight cannot be attributed to increase in intake of sugar or any other single food stuff. The remedy is not a motivated decrease in the intake of sugar, but rather a revision downward of the total diet after 40 with increase of physical exercise.

TABLE 5.—*Relation of Death Rate from Diabetes to the Consumption of Sugar in the United States, Great Britain and France*

Year	Death from Diabetes Rate per 100,000 in the United States	Consumption of Sugar in Pounds per capita in the United States	Death from Diabetes Rate per 100,000 in Great Britain	Consumption of sugar in Pounds per Capita in Great Britain	Death from Diabetes Rate per 100,000 in Paris	Consumption of Sugar in Pounds in France
1900	9.7	66.60	8.6	71.60	17.0	32.49
1901	10.3	69.70	9.1	76.33	....	.....
1902	10.4	72.80	8.4	66.47	....	.....
1903	11.3	70.90	8.5	60.29	....	.....
1904	12.9	75.30	9.3	70.35	....	.....
1905	13.0	70.50	9.3	63.34	....	.....
1906	13.0	76.10	9.7	69.35	....	.....
1907	13.9	77.54	9.7	70.76	....	.....
1908	13.9	81.17	10.3	69.49	....	.....
1909	14.4	81.80	10.4	72.40	....	.....
1910	14.9	81.60	11.0	70.20	18.4	40.41
1911	14.9	79.20	10.6	75.93	16.9	41.09
1912	15.0	81.30	11.1	74.69	17.7	41.46
1913	15.3	85.40	11.8	78.53	16.0	43.84
1914	16.2	84.29	12.2	72.36	14.3	25.86
1915	17.5	83.83	13.2	65.26	13.2	32.83
1916	17.1	79.34	13.2	56.38	13.4	31.68
1917	17.0	78.58	11.3	42.11	13.5	41.07
1918	15.8	73.36	10.6	45.70	9.6	13.28
1919	14.9	85.43	10.5	59.64	10.3	31.11
1920	16.1	86.56	10.0	45.04	....	39.29

Finally, while it has been pointed out that all evidence indicates a higher incidence of diabetes in the obese, there is, on the other hand, no evidence to prove that obesity is actually due to the abuse of sugar alone; for overweight is as common whether it is produced by the overuse of carbohydrates, of fats or of alcohol.

Table 5, taken from Emerson and Larimore, presents the relation of the death rate from diabetes to the consumption of sugar in the United States, Great Britain and France. It is clearly shown that there is a direct relation between the increase in the death rate from this disease and a rise in the consumption of sugar and vice versa.

26. Taylor: The Place of Sugar in the Diet, Hygeia **3**: 627 (Nov.) 1925.

CONCLUSIONS

As a result of our observations, as well as of those of Emerson and Anders, the following conclusions may be drawn:

1. There has been a marked increase in the death rate and incidence of diabetes in recent years.

2. The increase in the incidence of this disease, while it has included persons of all ages, has revealed itself most markedly in women at all ages and in men over 45.

3. The incidence of diabetes is proportionately much greater among the Jewish race. This susceptibility has been variously estimated as being from two to four times as frequent as among the rest of the population.

4. The cause of the greater susceptibility to this disease has as yet not been altogether satisfactorily established. Among the more frequent explanations given are: lack of proper physical exercise, unsuitable dietary habits, undue nervous strain and marked tendency to obesity, following the forty-fifth year of life. Without doubt, obesity plays by far the most important rôle. In this connection, Joslin has called attention to the important fact that the major number of casual factors referred to can actually be explained on the basis of obesity.

5. The relation of obesity to diabetes becomes more evident in the study of blood sugar tolerance curves in obese persons. In 57 per cent of Paullin's cases a lowered blood sugar tolerance was noted, and the proportion was 68 per cent in our obese Jewish patients. A certain number of these cases were detected in persons with early cases of diabetes. The variations between Paullin's and our own figures may perhaps be emphasized as further evidence in favor of the theory that there is a greater tendency to diabetes in obese Jews than in persons who are not Jews.

6. The increase in the consumption of sugar during the past years has been assumed to be a factor in the production of obesity and, therefore, of diabetes. There is no evidence, however, to prove that the overuse of sugar alone tends to produce this condition. Obesity can be produced as readily by the inordinate use of carbohydrates, fats or alcohol. It is likely that the overuse of food in general, together with lack of proper exercise, has been the most important cause of obesity, and consequently a direct factor favoring the development of diabetes.

7. Finally, as a result of these observations, it is apparent that in order to lessen the incidence of diabetes, the dietary habits of persons with a tendency toward obesity should be regulated, and adequate exercise should be instituted. This is especially important in persons past 45, and even more important among the Jewish population.

EXPERIMENTAL UREMIA*

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In previous papers,[1] it was shown that diuresis would not occur in animals suffering from acidosis as evidenced by a low alkali reserve (Van Slyke) even under the extreme provocation of the injection of 25 cc. of a 5 per cent solution of sodium chloride per kilogram of body weight. The following experiment is illustrative of what happens in more extreme acidosis.

EXPERIMENTS ON DOGS

Sodium chloride in the dosage mentioned was injected for ninety minutes into a dog weighing 17 kilograms. In this experiment the flow of urine lagged far behind the rate of injection, so that when the animal died after two and one-fourth hours, his water balance was 595 cc. plus. This failure of diuresis will result from acidosis of various kinds. The types that I have noted as demonstrated in experimental work include: (1) starvation acidosis, (2) exhaustion acidosis, (3) acidosis caused by acute respiratory infections, (4) acidosis resulting from the injection of hydrochloric acid through stomach tubes, (5) acidosis following ether anesthesia and (6) acidosis produced by the absorption of the fluid from autogenous edema. The latter will be exemplified in the following experiment.

Early in the work on this subject, one of the dogs had been tied too tightly to the table so that the leather thongs obstructed the circulation of one paw to such an extent that it became edematous. When this constriction was loosened and the paw massaged, there was a sudden cessation in the flow of urine. This was interpreted to mean that there was a pouring out of considerable fluid into the general circulation from tissues in a high degree of asphyxia, and that the resultant acidosis brought about a fixation of all the free water in the body.

Further experiments of this nature showed that this was unquestionably the case. In a previous communication,[2] it was shown that under a given stimulus the amount of water which it was possible to cause a dog to excrete was directly proportional to the carbon dioxide value (alkali reserve).[3] With this in mind, a dog was selected in which

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1. Andrews, E.: Water Metabolism I., Arch. Int. Med. **37**:82 (Jan.) 1926; Water Metabolism II., Arch. Int. Med. **37**:559 (April) 1926.

2. Andrews, E. (footnote 1, second reference).

3. Van Slyke, D. D., and Cullen, G. E.: Studies of Acidosis, J. Biol. Chem. **30**:289 (June) 1917.

the carbon dioxide combining power was 50, and he was injected with 5 per cent sodium chloride at the rate of 25 cc. per kilogram of body weight per hour for ninety minutes. Previous experience had shown that such a dog would be dehydrated to the extent of about 55 cc. per kilogram of body weight, in accordance with table given in a previous publication. His leg was tied tightly with a leather thong just before the injection was begun and at a point marked "x" in the curve; the

TABLE 1.—*Failure of Animal Given Sodium Chloride for Ninety Minutes to Dehydrate*

Time	Intake Sodium Chloride Solution Cc.	Urine	Water Balance Cc.	Comment
15	...	...	.....	Injection begun, carbon dioxide 38.
45	80	10	Plus 70	
75	160	10	Plus 150	
105	240	20	Plus 220	
135	320	23	Plus 297	
165	400	35	Plus 365	Convulsions
195	480	40	Plus 440	
225	560	50	Plus 510	
255	640	60	Plus 580	
285	720	62	Plus 658	Injection stopped, chills
315	...	70	Plus 650	
345	...	125	Plus 595	Dyspnea, died
360	...	125	Plus 595	

TABLE 2.—*Effects of Leg Tie*

Cc. 5 per cent sodium chloride per kilogram of body weight injected for ninety minutes

Time Minutes	Intake Sodium Chloride Solution Cc.	Urine Cc.	Water Balance Cc.	Temp.	Carbon Dioxide	Comment
30	...	...	...	...	50	Injection begun
70	40	12	28	...	....	
90	85	28	57	...	....	
100	125	62	63	...	....	
110	160	125	35	...	48	
120	200	150	50	...	38	Leg tie loosened
130	250	162	88	...	....	
70	290	166	124	...	....	
80	335	180	155	...	....	
90	375	182	193	...	....	
100	...	188	187	...	....	
110	...	188	187	..	....	
120	...	195	180	...	....	
130	...	195	180	...	.. .	
140	...	197	178	...	....	
150	...	197	178	...	23.9	Died

constriction was loosened and the leg vigorously massaged. The result as seen in table 2 was an almost immediate stoppage in the flow of urine. In this case the carbon dioxide fell ten points in eight minutes.

Numerous other experiments have shown that the fall in carbon dioxide value brought about by such constrictions varies between five and fifteen points. The fall is sharp in normal animals in which the danger point is not reached and does not continue, but reaches a maximum in a few minutes and returns rapidly to normal.

The nature of this acidosis was studied exhaustively, and other toxins besides the acid in the tissues could not be demonstrated. About seventy experiments gave negative results. These included transfusion of blood into other animals, injection of blood from edematous legs, injection of filtered edema fluid, extracts of alcohol and ether, filtered ground muscle and skin extracted with salt solution. Amino-acids could not be demonstrated in the fluids in appreciable amounts. Injections of lactic acid, however, gave similar results, and it seems justifiable to conclude that incomplete oxidation of glucose in the tissues was the offending feature. Clausen [4] has arrived at similar conclusions in the study of acidosis in infants. This substance is rapidly oxidized or reconstituted into higher molecules, and thus the rapid recovery is to be explained.

It is evident that in such an experiment many of the chemical features of uremia have been reproduced artificially. The organism is acid, and hydremia, increased chlorides and suppression of urine occur.

TABLE 3.—*Table of Leg Ties* *

Time Minutes	No. 1	No. 2	No. 3	No. 4	No. 5
0	54.2	48.6	47.8	38.0	44.8
5	....	43.8	....	38.6	....
10	39.0	....	42.4	....	....
15	....	44.8	42.6	35.8	39.6
20	....	....	....	40.2	....
30	47.8	47.0	43.2	....	40.6

* Leg released at 0 time and leg massaged vigorously

Therefore the study was continued, but such overwhelmingly large doses were not used. From 15 to 20 cc. per kilogram of body weight of a 5 per cent solution of sodium chloride was injected into a large series of animals (forty-seven) in which suppression of urine was brought about by leg ties, and pictures were produced in nearly half of them which were strikingly similar to that of uremic coma, the so-called "Kussmaul picture." These common factors may be grouped as follows:

1. Marked muscular irritability, followed by convulsions and vomiting; later muscular relaxation and death after many hours or days in coma.

2. Rise in blood pressure followed by marked fall in the later stages.

3. Increased respirations and later pronounced air hunger with forced inspirations involving the accessory muscles, and often typical Cheyne-Stokes breathing.

4. Watery polyuria subsiding into an oliguria with albumin, casts and high specific gravity.

5. Occasional extreme hyperpyrexia in the terminal stages.

4. Clausen, S. W.: Anhydremic Acidosis due to Lactic Acid, Am. J. Dis. Child. **29**:761 (June) 1925.

6. Rise in the nitrogenous elements in the blood.
7. Terminal edemas.
8. "Wet brain;" edema of the central nervous system.
9. Disturbed sugar metabolism with terminal glycosurias.
10. Carbon dioxide progressively lowered.
11. Absence of any pathologic renal condition indicating acute changes.
12. Hydremia.
13. Increase in the blood and tissue chlorides.

The data in table 4 cover the clinical features. Shortly after the injection is begun, there are slight fibrillary twitchings of the muscles. A pronounced rise in the blood pressure takes place; it generally rises to over 200 mg. of mercury within a short time. Severe clonic convulsions come on rapidly and occur every few minutes; this stage

TABLE 4.—*Artificial Uremia*

Male dog weighing 16 Kg.

Time	Intake	Urine Cc.	Albumin	Casts	Specific Gravity	Blood Pressure	Comment
0 min.	..	..	..	..	.....	120	Injection begun
30 min.	45	10	0	0	10.15	130	
60 min.	90	22	0	0	10.10	120	Muscular twitchings
90 min.	135	40	0	0	10.04	140	Leg tie loosened
120 min.	180	42	trace	0	.....	170	Convulsions
150 min.	225	..	0	0	.....	200?	Convulsions, dyspnea
180 min.	270	45	trace	..	.....	200?	Violent convulsions, injections stopped
5 min.	...	46	+	..	.....	200?	Incoordination
8 min.	...	..	+	..	.....	200?	Confused, restless
9 min.	...	..	..	..	.....	200?	
4 hrs.	...	50	−+	few	.....	180	Drowsy but easily aroused, one convulsion
8 hrs.	...	50	.	..	.....	160	Respirations 28, deeper coma
9 hrs.	...	57	+++	few	.....	100	Respirations 30, cannot be aroused
20 hrs.	...	..	++	..	.....	....	
24 hrs.	...	70	..	..	10.21	78	Completely comatose, respirations 34, pulse 130
32 hrs.	...	74	..	..	.....	....	Typical Cheyne-Stokes respiration 30 second intervals
56 hrs.	...	..	++	many	.....	50	

generally lasts from one to one and a half hours. Observations of any degree of accuracy on the blood pressure are impossible during this period, but it seems to remain well over 200. Vomiting often accompanies the convulsive stage. Slight stimuli bring on these convulsions.

After from one to two hours there is a period of drowsiness between the convulsions. In some cases there is a lucid interval between the time the convulsions end and the coma begins, and in others convulsions occur in the earlier stages of the coma. In these early stages there is a markedly increased flow of urine which is of low specific gravity and contains no albumin but occasional casts. Several hours after the beginning of the experiment the dog is generally in deep coma, from which he cannot be aroused. There is little or no change in this condition until death, which may occur in from four hours to three days after the beginning of the coma. During the later stages there is a fall

in blood pressure, suppression of urine or extreme oliguria and large amounts of albumin and casts; air hunger is pronounced. The respiration rises to from 30 to 40 and is deep. The accessory muscles of the thorax are brought into play and the alae nasi are active. Cheyne-Stokes respiration is common, and intervals between the groups of breaths may be as long as thirty seconds. The Kussmaul picture is accurate in every detail.

Fever as a terminal phenomenon occurred sporadically in the series. It generally rose from one to two degrees during the convulsive stage, and this rise was maintained until about the end of the period of coma, when a further extreme rise might occur. Temperatures of from 107 to 109 F. were usual, but extreme hyperpyrexias were not observed, such as were reported by Woodyatt [5] (126 F.) or myself [6] (120 F.), in severe dehydrations. As a matter of fact, there is an actual lessening in the amount of circulating fluid in the later stages of our experiments, as is shown by the rise in the refractometric index [7] of the blood at this time (table 8); without doubt this is an element in the case. It seems reasonable, however, to assume that a more important factor is the fixation of all the water in the body in a firm combination with proteins at this stage of the experiment, as the acidosis is extreme. In other words, in spite of an apparent hydremia, all the water in the body is "fixed," and "free" water is totally absent; this "free" water is necessary in order to allow the heat regulating mechanism to function. Under either of these assumptions, the fever can be classed as dehydration fever. If Fischer's [8] experiments on colloids are accepted, it is clear that the p_H range of the tissues under such circumstances would bring about the fixation of all the free water which an organism could possibly contain, and with a carbon dioxide value as low as 15, many gallons more would be held if it could be introduced into the body. Thus he has shown that if frog's muscle is immersed in 110 cc., it swells 41 per cent, but if it is immersed in 110 cc. distilled water plus 0.25 cc. tenth normal hydrochloric acid, it swells 51 per cent. This change in reaction is comparable to the physiologic changes and is sufficient to vary the volume 25 per cent (Fischer sec. 1, p. 80). It is obvious, therefore, that the fixation of fluids in the tissues would be complete with relatively slight degrees of acidosis.

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5. Balcar, J. O.; Sansum, W. D., and Woodyatt, R. T.: Fever and the Water Reserve of the Body, Arch. Int. Med. **24**:116 (July) 1919.

6. Andrews, E.: Water Metabolism III, Dehydration Fevers, Arch. Int. Med. **37**:815 (June) 1926.

7. Reiss, E. R.: Der Brechungskoefficient des Blutserums als Indikator fuer Eiweissgehalt, Inaug., Strassbourg, 1902.

8. Fischer, M. F.: Edema and Nephritis, ed. 3, London, John Wiley & Sons, 1921.

PATHOLOGIC CHANGES

Nitrogen.—In this series of animals with artificial uremia the nonprotein nitrogen (Folin-Wu [9]) averaged 95 mg. per hundred cubic centimeters of blood. Haden and Orr [10] examined the blood of 200 normal dogs, and the average nonprotein nitrogen was 30.8 mg. In only 11 per cent was it over 40, and the highest was 78. Even these figures are probably rather high, as the average carbon dioxide (Van Slyke) of the series was 34, and in half of the dogs it was below 40. In the laboratory of the University of Illinois, dogs that are well nourished and in good condition have almost always had carbon dioxide values over 40, so that one is led to conclude that the animals used by Haden and Orr were not in the best of condition. The nonprotein nitrogen content in this series (95 mg.) was not as high as one often finds in uremic coma, but it is now generally understood that retention of waste nitrogen is not the cause of the toxemia in uremia. Beaumont and Dodds [11] report four cases of typical uremic coma in which the blood urea never rose over 40 mg. per hundred cubic centimeters. Hogeneng and Florence [12] have shown that fish will live and thrive in 2 per cent solutions of urea. Folin and Dennis [13] have found high urea contents in the blood of cats. In 1890, Von Schroeder [14] found 2.6 per cent urea in the blood of the dogfish; these observations were later confirmed by Rodier.[15] Dennis [16] showed that in all elasmobranch fishes the urea content of the blood was from 800 to 1,000 mg. per hundred cubic centimeters. Fearon [17] says that urea is not toxic in the ordinary sense, and Herter [18] showed that the enormous concentration of 1 per cent is necessary in the blood of mammals before any untoward symptoms developed. In such high percentages, its action is that of disturbing the osmotic equilibrium of the tissues.

9. Folin, O., and Wu, H.: A System of Blood Analysis, J. Biol. Chem. **38**:81 (May) 1919.

10. Haden, R. L., and Orr, T. G.: Chemical Findings in the Blood of the Normal Dog, J. Biol. Chem. **65**:479 (Sept.) 1925.

11. Beaumont, G. E., and Dodds, C. E.: Recent Advances in Clinical Medicine, London, P. Blakiston's Son & Co., 1924.

12. Hogeneng, L., and Florence, G.: Bull. Soc. chem. de biol. **3**:174, 1921.

13. Folin, O., and Dennis, W.: J. Biol. Chem. **11**:87, 1912; Folin, O.: J. Biol. Chem. **14**:89, 1913.

14. Von Schroeder, quoted by Fearon: Physiol. Rev. **6**: 429 (July) 1926.

15. Rodier, quoted by Fearon: Physiol. Rev. **6**: 429 (July) 1926.

16. Dennis, W.: Nonprotein Organic Constituents in the Blood of Marine Fishes, J. Biol. Chem. **54**:693 (Dec.) 1922.

17. Fearon, W. R.: The Biochemistry of Urea, Physiol.Rev. **6**:429 (July) 1926.

18. Herter, C. A.: On Urea in Some of Its Physiological and Pathological Reactions, Johns Hopkins Hosp. Rep. **9**:617, 1899.

The enormous uric acid values [19] often found in the blood in gout are ample proof that it is not toxic in the amounts noted in uremia. More important, however, is the demonstration by Folin [20] that uric acid does not accumulate in the blood of nephrectomized dogs.

In view of these facts, it is not surprising that during the short time these experiments were carried on there was not a further increase in the nonprotein nitrogen to the enormous values often seen in uremia. The absence of such an accumulation strengthens the hypothesis that these waste nitrogenous products have little bearing on the subject of uremia. This point will be further discussed under the heading, "Pathologic Changes in the Kidneys."

Edema.—Gross edema occurred only twice in the series, which is not surprising in view of the comparatively short duration of the experiments. When one considers, however, that but 2 per cent at most and often less than 1 per cent of water is added to the organism, it is clear that this edema is not simply a matter of superhydration, but that it represents a change in the allocation of water in the tissues. Unfortunately, the gross specimens were not successfully photographed, but photomicrographs of the muscle tissue in the legs showed marked evidence of edema in and about the muscle fibers (fig. 1).

Most of the dogs in the present series showed a marked edema of the lungs clinically in the later stages of the uremia. This was manifested by moist râles and expectoration of large amounts of frothy mucus. Figure 2 is a photomicrograph of such a lung and shows the edema of the walls of the alveoli.

From an examination of table 7 it is clear that much of the protein in the blood has left the vascular system and is in the tissues. Magnus [21] has previously demonstrated that as much as 28 per cent of the protein might leave the capillaries after injections of salt, although the capillary walls are ordinarily impermeable to protein. He assumed that salt makes them permeable. Elliger and Klein [22] have pointed out the importance of protein that has left the vessels in the further binding of the fluid in the production in tissue edema. Table 8 also shows that the chlorides leave the vessels for the tissues in the terminal stages, and one can therefore assume that in the condition under consideration

19. Folin, O.; Berglund, H., and Derick, C.: The Uric Acid Problem, J. Biol. Chem. **60**:361 (June) 1924.

20. Folin, O.: The Nonprotein Nitrogen of Blood in Health and Disease, Physiol. Rev. **2**:460 (July) 1922.

21. Magnus, R.: Vergleich der diuretischen Wirksamheit isotonischer Salzloesungen, Arch. f. exper. Path. u. Pharmakol. **64**:68 (Aug.) 1900.

22. Elliger, H., and Klein, A. F.: Quellungdruck der Eiweisskoerper und Diurese, Arch. f. exper. Path. u. Pharmakol. **91**:1 (Oct.) 1921.

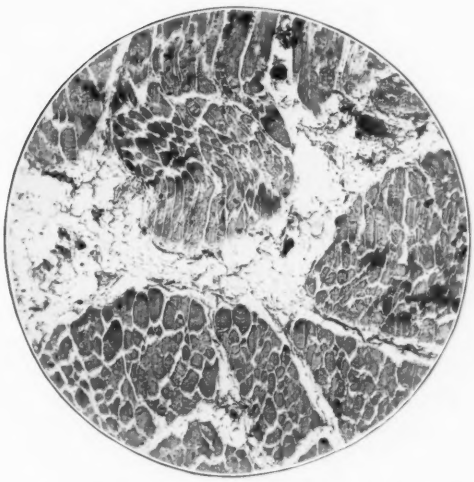

Fig. 1.—Edema of muscle tissue from the leg, showing separation of fibers and loss of striation.

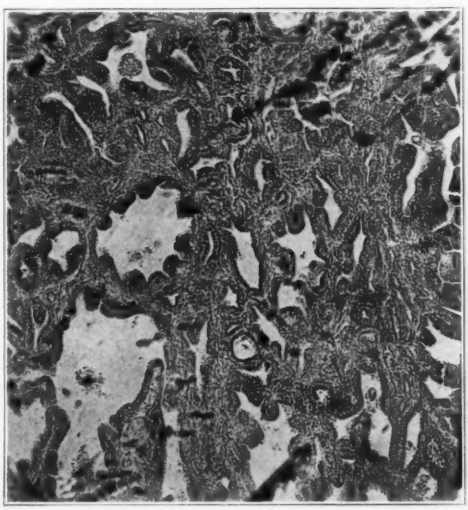

Fig. 2.—Edema of alveolar walls of the lungs.

the greater part of the fluid is no longer in the blood vessels but has made its way into the tissues. The rapidity with which sodium chloride will do this has been well demonstrated by Sherrington and Copeman,[23] who injected one third of the blood volume of 75 per cent sodium chloride during fifteen seconds and drew blood at the end of forty-five seconds. They found that 15 per cent of the injected sodium chloride had already passed into the tissues.

Pathologic Changes in the Brain.—Fischer states that many of the clinical phenomena of uremia are due simply to edema of the central nervous system. He showed that an increase of 10 per cent by weight constituted a fatal edema of the brain. Postmortem examination of six

Fig. 3.—Brain of dog with artificial uremia, showing edema and spreading of fibers of white matter.

of the animals in this series showed a typical "wet brain." Figure 3 illustrates the hyperemia of the nervous tissues and the edema in the white substance of the brain causing a separation of the nerve fibers. Attempts were made to study the eyegrounds in these cases, and the characteristic blurring of the outline of the disk and edema hiding the retinal vessels was seen. A diminished intra-ocular tension is characteristic of the later stages of human uremia, and in the animals in this series it was several times so marked that the anterior chamber of the eye was actually collapsed and the cornea concave. Measurements of the intra-ocular tension made by the use of a water manometer

23. Sherrington and Copeman: Blood Volume, J. Physiol. **14**:52, 1893.

connected with a needle inserted into the anterior chamber of the eyes of comatose animals showed that the tension had fallen practically to zero. The normal is said to be from 15 to 25 mm. (May).[24]

Glycosuria.—A profound disturbance in the carbohydrate metabolism was noted in all cases that were studied. Several animals had sugar in the last few drops of urine passed. Estimations on the blood sugar and lymph sugar made it clear that had total suppression of urine not been the rule, glycosuria would have been a common occurrence, as the urinary threshold was often exceeded. Similar terminal glycosurias are well known in clinical experience, and therefore one is justified in assuming that they are not due to a leaky kidney but to hyperglycemia. Table 5 illustrates this point. In every case there

TABLE 5.—*Sugars in Uremia*

Time	Lymph 1	Lymph 2	Lymph 3	Blood (average of 3)
0 Injection Begun	129.6	168.0	97.2	106
10 min.	139.6	209.4	94.6	...
20 min.	133.6	.....	97.2	...
30 min.	134.6	185.0	92.1	...
40 min.	125.1	197.8	83.4	127
50 min.	175.1	203.4	92.1	...
60 min.	159.1	.....	92.0	162
70 min.	166.9	209.4	89.7	...
80 min.	.....	.....	94.8	...
90 min.	159.1	215.9	106.0	204
100 min.	.....	.....	112.8	...
110 min.	175.0	246.0	120.6	...
120 min.	218.0	.....	126.0	...
150 min.	**331.8**	**235.0**	145.8	193
3 hrs.	.....	.....	**116.0**	...
6 hrs.	.....	.....	.....	230
18 hrs.	.....	.....	.....	240
24 hrs.	.....	.....	.....	227
30 hrs.	.....	.....	.....	208
36 hrs.	.....	.....	.....	140

was a rise in the blood or lymph sugar, and this generally exceeded 200 mg. per hundred cubic centimeters. In cases in which enough urine was passed to make a test, it contained sugar (table 5).

It had been assumed previously that the reason a patient with nephritis passed sugar in his urine just before he died was that his kidney allowed it to pass. This view is clearly wrong, as hyperglycemia is constant. The explanation of this hyperglycemia is simple. Glycogen stains of sections of the livers of the animals in this series made by the carmine method of Best,[25] showed that there was a total absence of glycogen in the later stages, whereas controls showed large amounts of stainable glycogen. In a previous publication[26] it was shown that

24. May, C. H.: Diseases of the Eye, New York, William Wood & Co., 1914.

25. Carleton, H. M.: Histological Technique, New York, Oxford University Press, 1926, p. 175.

26. Andrews, E.: Water Metabolism: IV. Sugar Metabolism in Dehydration, Arch. Int. Med. **38**:136 (July) 1926.

hydremia brought about a washing of glycogen out of the liver. Cajori [27] has shown that edema of a muscle blocks its utilization of glucose. Roche and Roche [28] also showed that glycolysis would not occur in the presence of marked acidosis. Schiff and Choremis [29] found that the hypoglycemia of fasting could be prevented by the administration of fluids. It is evident, therefore, that all the glycogen has been washed out of the liver and at the same time its utilization in the muscles has been prevented when acidosis and edema have been produced; therefore glycogen simply accumulates in the blood.

Acidosis.—A progressive fall in carbohydroxide occurs in every case. This happens not only during the injection of chloride but also continues after the injection has been stopped, and the carbon dioxide reaches as low levels as those often seen in uremic coma (table 6). This acidosis is not a feature of the so-called artificial uremia which is brought about by nephrectomy.

TABLE 6.—*Acidosis*

Carbon dioxide combining power (Van Slyke)

Time	
0 injection begun	42.8
1 hour injection ended	38.6
2 hours	37.0
3 hours	36.8
6 hours	30.2
11 hours	22.8
22 hours	20.0
25 hours	19.8
31 hours	15.6

Pathologic Changes in the Kidneys.—It is well known that one generally cannot correlate the clinical and microscopic pictures in uremia. Evidences of acute exacerbation of nephritis of long standing are usually absent. The changes seen are old, not acute ones causing suppression of urine. Neither suppression of urine alone nor double nephrectomy causes any of the clinical symptoms of uremia. There is, of course, a piling up of nitrogenous waste products in the blood, but never under any circumstances does the clinical picture of uremia arise. After double nephrectomy or ligation of both ureters, dogs live from four to ten days in a practically normal condition. Figure 4 represents a dog five days after double nephrectomy. He was active, played about the laboratory and had a good appetite. Such an animal dies rather

27. Cajori, F. A.; Crouter, C. Y., and Pemberton, R.: The Effect of Changes in the Circulation on Carbohydrate Utilization, J. Biol. Chem. **66**:89 (Nov.) 1925.

28. Roche and Roche: p_H Concentration and glycolysis, Hospitalstid. **69**:1002 (Oct. 14) 1926; abstr. J. A. M. A. **88**:138 (Jan. 8) 1927.

29. Schiff, E., and Choremis, C.: Exsiccation and Carbohydrate Metabolism, Jahrb. f. Kinderh. **114**:42 (Sept.) 1926.

Fig. 4.—Dog five days after double nephrectomy; no signs of uremia.

Fig. 5.—Normal kidney of dog with artificial uremia, showing tubules.

suddenly, apparently from inanition, without symptoms such as are ordinarily manifested in the terminal stages of nephritis. Clinical and histologic studies of such animals reveal none of the characteristic changes of uremia except the high nonprotein nitrogen. If, however, such an animal is injected with hypertonic salt solution in sublethal doses, the picture already described is promptly seen. These similarities hold good in the clinical, histologic and chemical changes described in this paper.

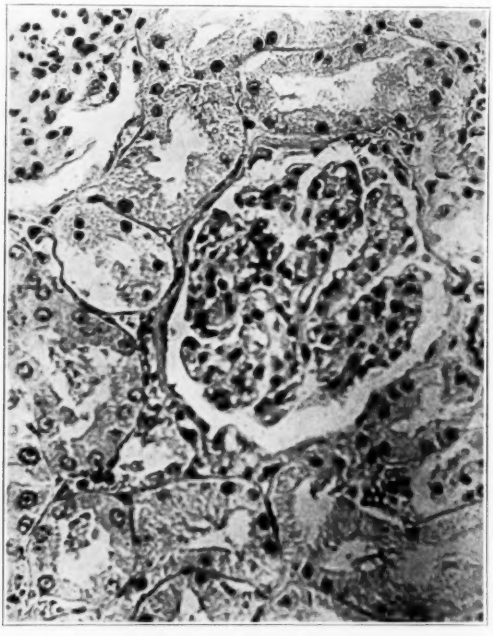

Fig. 6.—Normal kidney of dog with artificial uremia, showing glomerulus under high power magnification.

Scarcely any microscopic changes had occurred in the kidneys of the uremic dogs in this series. The glomeruli were invariably normal. The straight tubules and the loops of Henle were normal. Slight changes, certainly falling far short of those necessary to make a diagnosis of nephritis, were noted in the convoluted tubules; they were not extensive enough to cause blocking (figs. 5 and 6).

In this series there was a dog with chronic interstitial nephritis (fig. 7), and microscopic examination failed to reveal any new changes. Cloudy swelling, edema and glomerular degeneration were conspicuously

absent. These points lend great weight to the opinions of those who believe that the cause of nephritis is to be sought in a general metabolic disturbance and not in the kidneys. In these experiments, at least, it is clear that the kidneys were not responsible for the suppression of urine. The water and salts are fixed in the tissues, and suppression of urine can occur without histologic changes in the kidneys.

These results are in accord with the views already expressed by Fischer, namely, that edema is not due to renal obstruction. The clinical reports of Aldrich and McClure,[30] and Aldrich [31] are strong confirmations of this thesis. By means of the intradermal salt solution

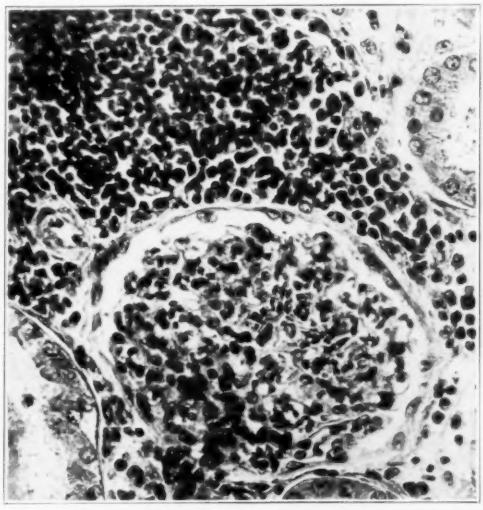

Fig. 7.—Artificial uremia, produced in old nephritic animal. Changes were all old. There was no cloudy swelling or acute degeneration.

test, they showed clearly that at the end of an attack of acute nephritis, the diuresis did not begin until the tissue thirst had definitely subsided. Aldrich [32] expressed similar views and elaborated on the mechanism in a more recent paper. Volhard [33] is also of the opinion that edema is

30. Aldrich, C. A., and McClure, W. B.: Time Required for the Disappearance of Intradermally Injected Salt Solution, J. A. M. A. **86**:293 (July 28) 1924; The Intradermal Salt Solution Test, J. A. M. A. **82**:1425 (May 3) 1924.

31. Aldrich, C. A.: The Clinical Course of Generalized Edema, J. A. M. A. **84**:481 (Feb. 14) 1925.

32. Aldrich, C. A.: The Treatment of Nephrosis, Am. J. Dis. Child. **33**:163 (Aug.) 1926.

33. Volhard, F.: Die doppelseitigen hematologischen Neirenkrankheiten, Berlin, 1918.

not due directly to renal lesions. Marriott,[34] Clausen[35] and many others who have studied the problem recently are in accord on this subject.

Water and Chlorides.—In the experiment with chlorides, while the injections continued there was a progressive rise both in the water and in the chlorides of the blood, but a fall in both began promptly as soon as the injections ceased (table 7). Table 7 is given to show that these injections did not cause the amount of chlorides in the blood and lymph to rise above the levels found clinically in uremia. This is a short experiment in which death occurred rapidly just about the time the amount of blood and lymph chlorides began to fall. Table 8 shows what happened in the later stages. In this case the suppression of urine was only partial, and relatively little water was added to the organism as a whole. The progressive fall in the blood and lymph chlorides after the injections stopped is shown clearly. The refracto-

TABLE 7.—*Chlorides*

Time Minutes	Intake	Urine	Urine Chlorides	Cl.	Blood Sugar	Protein	Lymph Chlorides	Comment
0	0	0	363	437	129	6.2	260	Carbon dioxide 2[illegible]
10	140	20	478	470	125	5.4	285	Injection begun
20	185	68	512	503	...	5.7	305	
30	225	71	542	477	125	5.1	289	
40	235	73	534	526	175	4.6	319	
50	270	76	537	528	159	...	322	
60	335	78	517	520	166	...	315	
70	430	81	538	526	...	...	319	
80	525	87	...	548	159	4.5	332	
90	610	..	...	569	...	4.6	345	Injection stopped
100	...	..	...	607	175	3.4	368	
120	...	..	...	642	218	2.9	389	
130	...	..	...	700	331	2.8	424	Carbon dioxide 1[illegible]
140	...	..	...	670	...	2.8	406	Died

metric readings and chlorides were taken from another animal; they illustrate how, in the later stages of the experiment, the chlorine made its way out of the blood while water passed from the blood into the tissues and caused anhydremia which probably explains the terminal dehydration fevers in these experiments. A count of the red blood cells and estimations on the hemoglobin confirm this. Table 9 shows what became of the chlorides. It is evident that the liver and brain have the greatest affinity for chlorides. This is a constant phenomenon checked many times and to a great extent explains why edema of the central nervous system is often a prominent feature clinically. Edema occurs in the brain before it occurs in any other organ, as is evidenced by the clinical phenomena and by the characteristics of the "wet brain" in cases in which edema cannot be demonstrated in other organs.

34. Marriott, W. M.: Parenchymatous Nephritis in Children, M. Clin. N. Amer. **7**:1413 (March) 1924.

35. Clausen, S. W.: Parenchymatous Nephritis, Am. J. Dis. Child. **29**:581 (May) 1925.

Pathologic Changes in the Liver.—I want to call attention to the most striking pathologic changes noted. As table 9 shows, there was a marked concentration of chlorine in the liver. In the more severe cases a peculiar change in the cells of the liver was found. They appeared to be literally falling to pieces (figs. 8 and 9). There was a tremendous swelling of the cells, and the more peripheral portions of the cytoplasm was actually frothy. Myriads of vacuoles were present in the cytoplasm, especially in the outer parts. A striking peculiarity

TABLE 8.—*Chlorides*

Time	Intake	Urine		Lymph		Blood *		Comment
		Vol.	Chlorides	Ref.	Chlorides	Ref.	Chlorides	
0	...	...	...	....	...	57	513	Carbon dioxide 31.0
30 min.	25	3	...	....	259	..	...	Injection begun
60 min.	80	...	...	....	...	..	...	
90 min.	130	...	...	....	...	..	...	
120 min.	210	24	336	....	257	..	...	
150 min.	280	70	507	....	...	..	...	
180 min.	350	137	525	....	257	..	...	
210 min.	420	213	554	40.0	...	..	...	
240 min.	480	296	563	41.0	269	..	...	
270 min.	495	352	557	37.5	...	..	...	Injection stopped
300 min.	...	375	623	36.0	352	..	...	
330 min.	...	391	647	35.0	...	..	...	
360 min.	...	400	654	35.5	334	..	...	
390 min.	...	408	714	34.5	334	..	...	
420 min.	...	410	721	32.0	325	..	...	
450 min.	...	...	...	31.5	...	..	...	
480 min.	...	411	375	29.5	330	..	...	
510 min.	...	...	...	31.7	305	..	...	Carbon dioxide 24.5
540 min.	...	...	...	35.5	307	..	...	Died
6 hrs.	...	...	...	....	...	42	814	
12 hrs.	...	...	...	....	...	47	660	
18 hrs.	...	...	...	....	...	51	527	

* From similar experiment

TABLE 9.—*Chlorides in Organs*

	Controls	Uremic Dogs		
		5 Hours	18 Hours	72 Hours
		Cc.	Cc.	Cc.
Blood	513	667	560	520
Muscle	309	338	297	503
Brain	412	462	536	825
Liver	495	338	495	783

is that the nuclei in every case were normal; only the cytoplasm took part in the degenerative changes. The pathologic change in the liver was the only real cellular degeneration that I noted in the experimental animals, and, as I shall attempt to show, was probably intimately connected fundamentally with the cause of the uremic toxemia.

COMMENT

From this series of experiments it is clear that the uremic syndrome in its entirety can be reproduced by the disturbance of the acid-base equilibrium and the mineral salt balance. According to the views of

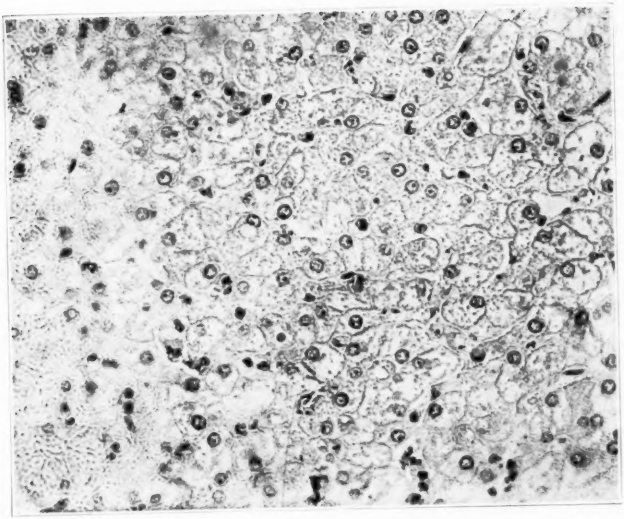

Fig. 8.—Degenerative changes in liver in artificial uremia under low power magnification.

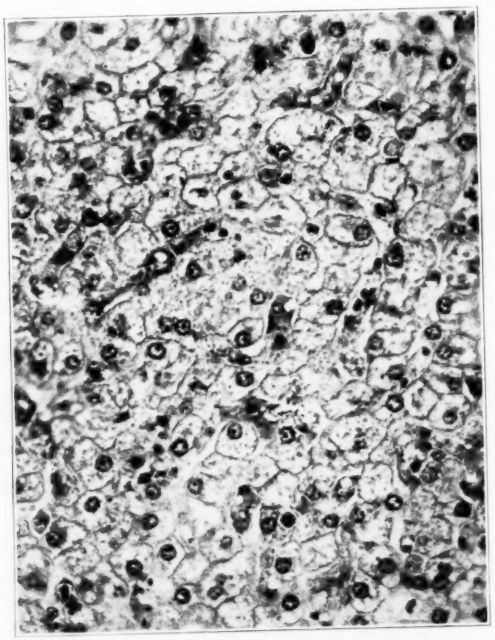

Fig. 9.—High power magnification of figure 8.

Macallum [36] and other biologists, life began in water without inorganic salts, and mammalian tissue fluids have the salt content of the Cambrian seas at the time when our ancestors left the sea. The membrane of the cell has developed as a protection against environments with different mineral content, and the nucleus has a membrane which is completely impervious to salts, as Macallum [37] has shown that the nucleus is free from inorganic material. This is interesting in view of the fact that in the present experiments the nucleus did not take part in the degenerative process. One is not surprised to find that the commonest disease of mammals, a disease which partakes of the nature of old age, should prove to be a breaking down of the metabolism of inorganic salts. Another interesting point in this connection is the demonstration by Reiss [38] that the nucleus of the cell is normal (p_H—7) while the cytoplasm has a p_H of from 5 to 6 due to carbon dioxide.

It has been shown by many workers whose material is well summed up by Hamburger [39] and by Jacobs [40] that this defense is a cell membrane which consists of a layer of emulsion of lipoids in which is contained the greater amount of the mineral salts in the cell. Pascucci [41] has made a membrane artificially by impregnating silk with lecithin and cholesterol and has shown that it would behave in many respects in the same manner as a living membrane. The permeability of this membrane is influenced by the relative concentration of potassium and calcium ions. Loeb [42] has shown that sodium favors the entrance of potassium ions into the cell. The evidence is now overwhelming that increases in the alkali metals bring about increases in cell permeability. The work of Jacobs, Loeb, Clewes, Harvey, Ostehout, Overton and many others has established this without a doubt. Cases of decreased permeability resulting from calcium are nearly as common. Besides the aforementioned writers, True, Bartlett, Laquer, Rosenow, Magnus and many others have contributed to this field. Hamburger [43] has also shown

36. Macallum, A. B.: The Palaeochemistry of the Ocean in Relation to Animal and Vegetable Protoplasm, Tr. Canad. Inst. **7**:535, 1904.

37. Macallum, A. B.: The Palaeochemistry of Body Fluids and Tissues, Physiol. Rev. **6**:316 (April) 1926.

38. Reiss, P.: Quelques données sur le p_H inferieur apparent du protoplasme et du noyon, Arch. de phys. biol. **4**:35, 1924.

39. Hamburger, H. J.: A Discourse on Permeability, Physiological and Pathological, Lancet **2**:1039 (Nov. 19) 1921.

40. Jacobs, H. M.: General Cytology, University of Chicago Press, 1924, p. 99.

41. Pascucci, quoted by Jacobs (footnote 40).

42. Loeb, J.: Sodium Chloride and Selective Diffusion in Living Organism, J. General Physiol. **5**:231 (Nov.) 1922.

43. Hamburger, R.: Ueber die Bedeutung der Kalium-und Calciumionen duer das kuenstliche Oedem und fuer Gefaessweite, Biochem. Ztschr. **129**:153, 1922.

that artificial edema may be produced by solutions deficient in calcium Thomas [44] has reproduced this phenomenon in vitro and has shown that the important point is not the concentration of either the potassium or the calcium ion but the relation between the two.

It has also been amply demonstrated by the work of Little, Overton, Troendle, Jacobs, Harvey, Bethe and others that increase in the hydrogen ion concentration brings about an increased permeability of cells. Of special interest in this connection are the experiments of Koeppe,[45] who showed that an increase of carbon dioxide rendered cells more permeable to chlorides. These observations have been confirmed by Henderson and Spiro.[46]

In view of these facts, it is clear that injections of chlorides into animals that were unable to get rid of them on account of acidosis or nephrectomy must have caused a tremendous increase in the permeability of the cells in the organ which holds the most chlorides, the liver. It is even within the range of probability that such an increase of permeability took place that the cells really fell to pieces. It has already been shown that the cells were no longer able to retain the carbohydrates which make their way into the blood.

The work of Andrews [47] and of Hewitt [48] has recently called attention to the fact that uremic patients have substances in their blood that give a positive diazo reaction (Ehrlich). Becher [49] is of the opinion that these originate in the tissues. They probably are partial oxidation products of protein. Blotner and Fitz [50] confirm these observations, and show that after nephrectomy in dogs, positive diazo reactions can be obtained in from twenty-four to forty-eight hours. In the artificial uremia in this series (table 10), while the control nephrectomized animals gave negative reactions throughout, those injected with hypertonic salt solution gave positive reactions within a short time. This is probably to be interpreted as a leaking into the circulation of partially oxidized nitrogenous products which are ordinarily held within the cells.

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44. Thomas, W. H.: Personal communication (work as yet unpublished).

45. Koeppe, H.: Physiologische Kochsalzloesung, Arch. f. d. ges. Physiol. **65**: 492, 1897.

46. Henderson, L. J., and Spiro, K.: Zur Kenntnis des Ionengleichgewichts in Organism Biochem. Ztschr. **15**:105, 1909.

47. Andrews, C. H.: Unexplained Diazo-Colour Reaction in Uremic Sera, Lancet **1**:590 (March 22) 1924.

48. Hewitt, L. F.: The Diazo Reaction in Uremic Sera, Biochem. J. **19**:171 (Feb.) 1925.

49. Becher, E.: Diazo and Urochromogen Reactions in Renal Insufficiency, Arch. f. klin. Med. **148**:10 (July) 1925.

50. Blotner, H., and Fitz, R.: The Diazo Tests in Nephritis, J. A. M. A. **88**:985 (March 26) 1927.

The origin of albumin in the urine has as yet not been demonstrated. One is tempted to entertain the possibility that the liver is the source in these cases. It is known that the kidneys are permeable to all proteins except blood proteins. If other proteins are injected intravenously, they are eliminated quantitatively. However, such a condition eventually injures the renal epithelium to such an extent that it becomes permeable to blood proteins as well. In view of the obvious increase in permeability in this case, and especially in view of the fact that it has been demonstrated that the liver allows both carbohydrates and soluble nitrogenous elements to escape, the suggestion that proteins leak from it into the blood seems plausible; the precipitation reactions of these urinary proteins are being studied according to the technic recently published by Welker, Thomas and Hektoen.[51]

The calcium content of the body cells is difficult to ascertain with any accuracy. Most of the studies on calcium in relation to permeability have been made on lower animals, or indirect methods have been used.

TABLE 10.—*Diazo Reactions* *

Time Hours	Dog A. (control)		Dog B.		Dog C.	
	Diazo	Carbon Dioxide Combining Power	Diazo	Carbon Dioxide	Diazo	Carbon Dioxide
0	0	39.2	0	42.0	0	44.4
1	0	....	0	....	0	....
2	0	....	0	....	positive	35.8
3	0	....	tr?	27.2	positive	....
4	0	....	positive	....	positive died	29.2
5	0	....	positive died	27.2	.....	....
8	0	40.3	.....	....	.....	....

* Ureters on 3 dogs ligated at 0 time. Dog A, control. Dogs B and C injected with 15 cc. per cent sodium chloride during first hour.

It is known that blood calcium has little relation to the calcium retained in the cell membrane in a protein lipoid complex. Injection of large amounts of calcium into the veins is followed by rapid elimination and must often be repeated to maintain a high blood level, and then one is never sure that the calcium is being incorporated into the tissues. The work of Hirshhorn and Maendl [52] on this phase of the problem leaves us in doubt as to the mechanism of its action. Walters [53] and his associates have shown that enormous doses of calcium are necessary to counteract the tendency to hemorrhage in obstructive jaundice; in fact, such large amounts that but infinitesimal proportions of a dose

51. Welker, W. H.; Thomas, W. H., and Hektoen, L.: Urinary Proteins, J. A. M. A. **86**:1333 (May 1) 1926.

52. Hirschhorn, J., and Maendl, H.: Wien. Arch. f. inn. Med. **4**:379, 1922.

53. Walters, W., and Bowler, J. P.: The Preoperative Preparation of Patient with Obstructive Jaundice, Surg. Gynec. Obst. **69**:200 (Aug.) 1924. Walters, W.: Preoperative Preparation of Patients with Obstructive Jaundice, Minnesota Med. **6**:25 (Jan.) 1925.

could make its way into the cell. It is clearly a case of application of the law of a mass action. Papers by Blum and others,[54] Lowenberg[55] and others, Rockwood and Barrier,[56] MacCallum,[57] Porges and Pibram,[58] and many others have noted the diuretic effects of calcium under certain conditions, especially in some types of edema, but the results are uncertain. Some brilliant results are reported, and in other apparently similar cases the effect is nil. The crux of the matter seems to be the difficulty to distinguish between the "transient" inorganic calcium and the calcium bound up in a protein lipoid complex.

Hetenye and Nogradi[59] have recently demonstrated that in nephritis there is a delay in the excretion of calcium salts. One might assume that in such cases it is needed. All studies in calcium metabolism are handicapped by the fact that calcium is so largely excreted by the bowel, and therefore measured with great difficulty; for that reason, only blood studies made over short periods of time in response to definite stimuli can be of much value. The important studies of Boyd and others[60] are subject to this criticism. They demonstrated an increase in calcium in the blood of nephritic children and a decrease in potassium. This might be assumed to mean that there was a pouring out of calcium from the tissues into the blood; since further increase of this calcium by injection is of benefit, it is difficult to interpret it any other way.

The experiments of Petersen[61] in anaphylaxis are an excellent example of the method which gives the real answer to the problem. He and his co-workers inserted a cannula into the lymph duct of dogs and measured the calcium produced by the injection of bacterial protein during shock. Their work showed clearly that there was a sharp rise in the calcium and a corresponding fall in the potassium in the lymph.

54. Blum, L.; Aubel, E., and Hausknecht, R.: L'action diuretique des sels de calcium dans les edemas générales, Bull. et mém. Soc. méd. d. hôp de Par. **2**:1501, 1921.

55. Lowenberg, H.: L'action cardiotonique et l'action diuretique du chlorure de calcium, Ann. de méd. **13**:172, 1925.

56. Rockwood, R., and Barrier, C.: The Calcium Treatment for Edema, Arch. Int. Med. **33**:643 (May) 1924.

57. MacCallum, J. B.: The Influence of Calcium and Barium on the Secretory Activity of the Kidney, Univ. of Calif. Publications (Physiol.) **2**:31, 1904-1905.

58. Porges, O., and Pibram, E.: Ueber den Einfluss des Calciums auf Diurese, Arch. f. Exper. Path. u. Pharmacol. **59**:30 (June) 1908.

59. Hetenye, G., and Nogradi, S.: Calcium Excretion Through the Normal and Diseased Kidney, Klin. Wchnschr. **4**:1308 (July 2) 1925.

60. Boyd, G. L.; Courtney, A. M., and MacLachlan, E.: The Metabolism of Salts in Nephritis, Am. J. Dis. Child. **32**:29 (July) 1926. Boyd, G. L., and Courtney, A. M.: The Metabolism of Salts in Nephritis, Am. J. Dis. Child. **32**: 197 (Aug.) 1926.

61. Petersen, W. F., and Hughes, T. P.: Inorganic Alterations of the Lymph in Anaphylactic Shock, J. Biochem. **63**:179 (March) 1925.

which can only be interpreted to mean that the calcium was being disposed of by the cell and the potassium absorbed, which is in complete accord with the known factors of the permeability in such cases. This is the first answer to the question in unequivocal terms. There can be no other interpretation than a fall in the cell calcium and a rise in the cell potassium corresponding to sudden great increase in permeability.

Three such experiments were made on animals in which uremia was induced, and definite results were obtained. The curve in fig. 10 shows the average of the three. It is definitely shown that there is a sudden increase in the amount of calcium in the lymph coming from the cells, which are becoming more permeable. This coincides with the injection of sodium chloride.

With these points in view, attempts were made to induce uremia by the injection of Locke's solution, which has the proper calcium-potassium ratio. The syndrome produced was not so severe or prompt in its onset, but nevertheless was typical. With the work of Walters in

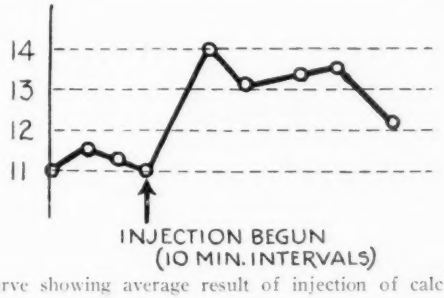

Fig. 10.—Curve showing average result of injection of calcium in artificial uremia.

mind, injections of hypertonic salt were made as before; when the convulsions were at their height and further injections of an overwhelming dose of calcium were given (1 Gm. calcium in a dog weighing 8 Kg.). The results were surprising. There was a prompt cessation of convulsions, urination began, and the dog made a complete recovery. It is clear that the numerous therapeutic attempts to use calcium have a logical basis, but enormous doses are necessary. It is doubtful whether oral administration can ever be of any value. All the dogs used in the experiments were fed bone ash as a routine, and this did not have any effect. It is the organic calcium which is needed, and overwhelming doses are necessary to bring into play the law of mass action to bring calcium rapidly into cells. The favorable results following the administration of parathyroid extract reported by Mason [62] are

62. Mason, E. H.: A Case of Chronic Nephritis Treated with Collip's Parathyroid Extract, Canad. M. A. J. **16**:538 (May) 1926.

suggestive; it is noteworthy that the increase in blood calcium was slight, much less than can be induced by small doses given intravenously, but the increase represents organic calcium and the method is therefore effective.

SUMMARY

If animals in which suppression of urine is brought about by either nephrectomy or acidosis are injected with hypertonic salt solution in amounts such that the chloride content of the blood is comparable to that in uremia, they pass into a condition closely resembling uremia. Every manifestation of uremia seen in the human being is reproduced. This is true of the histologic and chemical factors as well. High blood pressure, convulsions followed by coma, "wet brain" and suppression of urine are cardinal points.

Both the chlorides and water rapidly make their way from the blood into the tissues. The brain and liver have the highest chloride contents, and are the organs chiefly involved. There is marked degeneration in the liver which is shown to be due to an increased sodium-calcium ratio which renders the liver cells permeable.

One seems justified in assuming that these degenerative changes in the liver are the fundamental source of the uremic toxins, that albuminous substances are allowed to escape into the blood on account of the destruction of the cell membranes, and that this is the source of the albumin in the urine in nephritis.

CONCLUSIONS

1. All the clinical, chemical and histologic features of uremia may be reproduced by disturbing the mineral metabolism, even in the presence of normal kidneys.

2. Recent clinical attempts at calcium therapy in nephritis have ample experimental basis, but enormous doses will be needed, and a distinction must be drawn between transient calcium and calcium actually incorporated into the body cells.

Book Reviews

MEMORIAS DO INSTITUTO DE BUTANTAN. Volume 1, no. 1, 1918; volume 1, no. 2, 1918-1919; volume 2, no. 1, 1925; volume 3, 1926. Addenda to the Memorias, volume 1, no. 1, 1921. São Paulo, Brazil: Inst. of Butantan.

These interesting volumes deal mostly with problems of tropical medicine. Much of the material for the studies is furnished by the poisonous spiders and snakes of the neighborhood of São Paulo. The scientific standards set in this institute of immunology are high. Much of the work consists of careful study and classification of the flora and fauna of the region, and each number contains monographs on the structure and biology of the poisonous snakes. These articles are always lavishly illustrated with drawings, photomicrographs of the poison glands and photographs showing the habits of the snakes. One whole volume (the Addenda) is given to a description of a small snake, *Lachesis*, a close relative of our rattlesnakes, which dwells almost entirely in trees and lives on birds. The venom of this reptile is fatal to birds if injected in amounts as infinitesimal as 0.00001 Gm. An exceedingly powerful antitoxin was prepared against this venom.

Volume 1, number 1, is mostly concerned with the flora and fauna of the region, and contains a long article on a genus of insectivorous plants and a series of histologic studies of the poison glands of several species of snakes. These biologic studies are important on account of the great specificity of the antitoxins and the need for rapid diagnosis concerning the source of the lesions clinically. For example, Dr. Vital Brazil, now director of the institute, reports in this number the preparation of a powerful antitoxin for scorpion bites. Even in this case its clinical efficiency varied greatly for different species of scorpions.

Volume 1, number 2, is noteworthy on account of an elaborate monograph on filariasis by Dr. Afranio Amaral. The various pathologic conditions are classified, studied and several types of filaria are found. The specificity of these types for different tissues and regions is well marked. A complete series of histories is given with the protozoal observations in each case. Thorough hematologic observations showed that there is a characteristic eosinophilia which is more pronounced during the night and a slight increase of small lymphocytes which becomes much more marked during the night. During the febrile exacerbations, there is a moderate leukocytosis of the polymorphonuclear type.

In considering the therapy of filariasis, one must remember that the first consideration should be directed against the organism. Chemotherapy has been disappointing. The one measure at command is radiotherapy; this has yielded encouraging results, especially in those lesions in which knowledge of the pathologic anatomy is sufficient to indicate to which parts to apply the rays. Surgery is of no avail in the acute stages and, if applied injudiciously, it may be the means of bringing about a dissemination of a localized disease. In those cases in which the filaria are already dead or the process has become stationary, "the cold cases," surgical measures give brilliant results. This is, however, definitely limited to the sequelae of filariasis. In such conditions operations to improve or to anastomose lymph channels (the Kondoleon operation) are generally insufficient per se. In addition, radical excisions of the lymphangiectatic areas are necessary, and the results are good. The tissue healing is surprisingly good in such edematous conditions.

Another interesting contribution in this number is Amaral's report on the healing of phagedenic ulcers under the influence of the local application of dried normal serum. This measure seems to be almost specific in such conditions; it is followed almost overnight by the disappearance of the offending spirochetal flora, and rapid cicatrization takes place. An adequate explanation of this phenomenon can not be found.

Volume 2, number 1, contains a report of the work of Brazil and Vellard on spider venoms; twenty varieties were studied, and specific antiserums were prepared. These are all highly specific, and therefore the recognition of the type of spider is important. The administration of antitoxins not only has the general effect of combating the neurotoxins but acts strongly in preventing or healing the enormous ulcers that result from such bites. The nature of these poisons is unknown, but they have certain peculiarities which sharply distinguish them from most others. They are not entirely destroyed by heat less than 100 C., and they are resistant to the action of chemicals.

Vaz's article on the immunization of animals by mouth against the Shiga bacillus is also well worthy of study.

In conclusion, one is led to regret that the material from this institute is not available in the American literature. Much of our fundamental knowledge of immunology was acquired by study of materials of the class here included, and one can not but feel that future progress will be made by the study of the immunity reactions of these substances which are far simpler than bacterial proteins.

The third volume of the report of the work done by Dr. Vital Brazil and his associates is an imposing one, and covers a wide field of studies in immunology. The major portion of the work, however, deals with the toxicology and biology of the tropical fauna of the neighborhood of São Paulo. The book is liberally illustrated with photographs, some in color.

Among the most noteworthy contributions is the study of Dr. Vellard on toxic secretions from the skin of toads. This interesting material is not a protein, it dries rapidly in air, can be redissolved in distilled water, saline solutions, alcohol, ether and chloroform. It resists without change temperature of 160 degrees, the action of light, time and strong acids and alkalis, with which it gives off color reactions. This substance is exceedingly toxic when introduced either parenterally or orally; it produces nausea and vomiting, followed by paralysis and violent convulsions and death from respiratory failure. The action on the heart is similar to that of digitalis, causing an augmentation of the force of the beat and stoppage in systole.

The previous reports of Vaz on the possibilities of immunization by mouth to the Shiga bacillus are continued and give great hope that this simple method may be of great use as a public health measure. The vaccine is made by killing a twenty-one day culture in bouillon with solution of formaldehyde. A dose of 20 cc. of this vaccine by mouth will completely immunize a rabbit against the development of experimental dysentery.

Next follows a continuation of the studies of the bufo toxin — a complete and elaborate experimental demonstration of the physiologic action of this interesting substance. It is shown to have a markedly exciting action on unstriated muscle tissue, and it can be demonstrated that this is not due to epinephrine content of the secretions as has been suggested by other authors. The third memoir on the toxins of the forms of tarantula found in the neighborhood of the São Paulo is also worthy of mention. There is wide variation in the toxic content of the gland of the various species of the spiders examined, but only two real venoms were found, and against these two exceedingly powerful antitoxin serums are produced which have been demonstrated in a large number of cases to have brilliant clinical results.

BASAL METABOLISM IN HEALTH AND DISEASE. By EUGENE F. DUBOIS, M.D., Medical Director Russell Sage Institute of Pathology, Associate Professor of Medicine, Cornell University Medical School, New York. Price, $5. Pp. 431. Philadelphia: Lea & Febiger, 1927.

In this second "thoroughly revised" edition of his book, DuBois states that he attempts to bring basal metabolism out of the realm of pure physiology into the domain of clinical medicine. The attempt is excellent, but the task is not an easy one. "The domain of clinical medicine" is by no

means a simple, homogeneous organization with one aim in view, nor is it easily understood. From the remote rural district to the clinic of a modern medical school is a long way. One cannot read this excellent book without considerable speculation concerning the response it would receive from the various sections of said "domain."

In the first part of the book there is much that is necessarily academic, especially from the standpoint of the clinician, and still he finds here such useful discussions as that in the second chapter, in which the physiology of carbohydrate, fat and protein digestion and the influences of these substances on metabolism are reviewed. Also "the factors which influence the normal basal metabolism" and "theories concerning the basal metabolism" are easily concise contributions to the clinician's daily needs in understanding the task that he attempts in caring for any group of sick people.

The second part of the book deals with metabolism in disease and for one not concerned with the technical side of these studies, it is more interesting and more useful than the first. What physician does not deal with the conditions "undernutrition" and "overnutrition and obesity"? The regulation of weight by appetite, the specific dynamic action of food, endocrine disturbances and the influence of heredity are important subjects considered under the subject of obesity.

In the chapter dealing with metabolism in diabetes the variations encountered are described and, as far as possible, explained. There is careful detail concerning the influence of various arrangements of diet on the metabolism of the diabetic patient. The results obtained by many groups of workers who have concentrated their efforts on this problem are extensively quoted.

There is a timely warning against placing too much emphasis on the metabolic rate alone in the diagnosis of disease of the thyroid. Naturally, the chapter dealing with disease of the thyroid is relatively long. In it is presented a usable summary of the important contributions that have been added to the literature of the subject through studies of metabolism.

The subject of fever is found most interesting and instructive. Here one finds clear discussion, based on quantitative determinations, of many symptoms and factors that are commonplace with clinicians and research workers. Overproduction of heat, diminished elimination of heat, changes that accompany chills and fever, relation of changes in metabolism produced during fever to changes produced by diet, are subjects clearly presented.

There is much else to commend in this book. When one has finished reading it, he feels that it has a definite value for clinician and investigator, and that the author has gone far toward accomplishing the purpose described in his preface. It is most appropriate that such a contribution should come from the Russell Sage Institute where so much earnest effort has been made in the study of metabolism.

BIRTH INJURIES OF THE CENTRAL NERVOUS SYSTEM. Part 1: Cerebral birth injuries and their results, by F. R. Ford of Johns Hopkins School. Part 2: Obstetrical injuries to the spinal cord by Bronson Crothers and Marion C. Putnam, Harvard Medical School. Price, $4. Pp. 220, with 70 illustrations. Baltimore: Williams & Wilkins Company, 1927.

Part one deals with the sources of intracranial hemorrhages occurring at birth as coming from (1) the small tentorial vessels, (2) vena magna galeni, which may rupture as the result of distortion during the molding of the head, (3) cerebral veins, namely, the superior longitudinal sinus, (4) in the severe types, from injuries of the superior longitudinal sinus, transverse sinus and straight sinus, (5) but rarely from injury to the choroidal veins, which occurs only in stillborn premature infants.

The author says further that hemorrhage is never extradural, unless associated with fracture of the skull. He also emphasizes the fact that an important contributing cause of intracranial hemorrhage is prematurity. In this con-

nection he quotes authorities who state that intracranial bleeding occurs sixteen times more frequently in premature than in full-term infants. The cutaneous vessels of premature infants are much more fragile. The skin of mature babies will withstand a pressure of 520 mm. of mercury while in premature infants a pressure of 150 mm. of mercury will cause a hemorrhage of the cutaneous vessels. Another interesting statement by the author is that he has never found a single instance in which a true cerebral diplegia was caused by intracranial hemorrhage at childbirth. A fair percentage of these cases are microcephalic. The paralysis in such cases is a perfect bilateral symmetry in over 95 per cent of the cases and cannot be related to injury at birth or meningeal hemorrhage.

The relation of injury at birth to hydrocephalis, to epilepsy and mental defect, is fairly well discussed, including case histories and anatomic observations.

Part two includes a discussion on injuries to the brachial plexus. The important causal factors as in injuries of the brachial plexus in adults is "traction" and not asphyxia or generalized pressure. The injuries to the plexus and spinal cord in practically all the cases are caused by traction during delivery. The monograph is most interesting and instructive and deserves a place in the library of the general practitioner who must include obstetrics in his practice.

TRAITE DE PHYSIOLOGIE, NORMALE ET PATHOLOGIQUE. Published under the editorship of Drs. G. H. Roger and L. Binet, Professors of Physiology in the Faculty of Medicine, University of Paris. Volume 11. Price, 65 francs. Pp. 496. Paris: Masson and Cie, 1927.

This volume is a composite production containing chapters on histogenesis of the sex cells, by Champy; sex physiology of the male, by Busquet; sex physiology of the female, by Vignes; secondary sex characters, by Pezard; physiology of pregnancy, by Vignes; physiology of the new-born, by Binet; physiology of lactation, by Porcher; one chapter on histology of growth, by Champy; another chapter on physiology of growth, by Lesne and Binet, and one on heredity and one on teratology, both by Rabaud.

As in similar monographs by several authors, the chapters have an unequal value. The monograph, as a whole, is reliable, helpful, and fairly comprehensive. The chapters by Dr. Vignes are particularly excellent. Dr. Pezard, who writes on the secondary sex characters, has done much experimental work on the physiology of sex and sex characters, particularly among birds. It is therefore surprising to find that this chapter is not up to date on many points. Dr. Pezard seems unfamiliar with the recent important work in this field by Drs. Moore and Oslund in this country. The other chapters contain valuable references to the world literature on the specific points under discussion.

The completed series will constitute a valuable work for reference.

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